Universidade de Aveiro Departamento de Biologia
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Métodos biológicos de controlo da amêijoa invasora *Corbicula fluminea*

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Aplicada, ramo Ecologia, Biodiversidade e Gestão de Ecossistemas, realizada sob a orientação científica do Doutor Bruno Castro, Investigador Auxiliar do Departamento de Biologia e CESAM, Universidade de Aveiro, e da Doutora Joana Pereira, Estagiária de Pós-Doutoramento do Departamento de Biologia e CESAM, Universidade de Aveiro.

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À minha avó Irene…

A “formiga rabiga” ainda inspira a sua neta.

o júri

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palavras-chave

Corbicula fluminea, impactos ecológicos e económicos, controlo biológico, predação, evitamento.

resumo

A amêijoa invasora *Corbicula fluminea* é causadora de graves impactos, tanto ambientais como económicos, nos locais invadidos. Os métodos de controlo químicos e físicos são especialmente dispendiosos e prejudiciais para o ambiente, atingindo espécies não alvo. O controlo biológico de espécies invasoras tem o potencial de ser uma ferramenta eficaz, segura, de baixos custos e benigna para o ambiente. No âmbito da presente dissertação, avaliou-se o potencial de utilizar peixes de água doce como ferramentas de controlo biológico, nas suas vertentes direta (predação propriamente dita) e indireta (indução de evitamento).

Em primeiro lugar, avaliou-se o potencial da predação como método direto de controlo biológico de *C. fluminea*. Numa primeira fase, foram inspecionados os conteúdos estomacais de peixes capturados num sistema de canais e valas onde *C. fluminea* é extremamente abundante. Apenas se confirmou a presença desta amêijoa nos estômagos de *Barbus bocagei*, com uma frequência de ocorrência de 50%, mas com uma reduzida importância (< 5% conteúdo estomacal). Com base nestes dados e na literatura disponível, foram selecionados dois modelos para experiências de predação com a presa *C. fluminea*: *B. bocagei* e *Lepomis gibbosus*. O interesse demonstrado por ambas as espécies de peixes por exemplares de *C. fluminea* nas experiências de predação foi muito reduzido, mesmo em amêijoas de reduzidas dimensões (< 1 cm). Experiências adicionais permitiram demonstrar que a parca utilização de *C. fluminea* como presa se deveu à ação protetora da sua concha. Do ponto de vista aplicado, o potencial destas espécies de peixe como predadores de *C. fluminea* é reduzido (*Barbus*) ou nulo (*Lepomis*), o que afasta a possibilidade de os utilizar como ferramentas de controlo direto.

Utilizando *L. gibbosus* como modelo experimental em laboratório, testou-se ainda os efeitos indiretos da presença do peixe no comportamento de *C. fluminea* através de experiências de evitamento. Estas experiências basearam-se na capacidade das presas modificarem o seu comportamento ao percecionarem sinais infoquímicos por parte do predador. Os resultados obtidos demonstraram que as amêijoas respondem à presença do predador, deslocando-se ativamente. Contudo, a amplitude e direção dessa deslocação dependeram da presença de um refúgio para a presa, da dieta dos peixes, e das condições de iluminação. Estes resultados demonstram a necessidade de compreender melhor estas interações, no sentido de estabelecer a utilidade do uso de predadores (ou os seus infoquímicos) como forma de criar zonas de exclusão em áreas sensíveis de infraestruturas hidrodependentes.

keywords

Corbicula fluminea, ecological and economic impacts, biological control, predation, avoidance.

abstract

The invasive clam *Corbicula fluminea* is the cause of severe impacts, both environmental and economic, in invaded sites. Chemical and physical control methods are particularly expensive and harmful to the environment, affecting non-target species. Biological control of invasive species has the potential of being an effective, safe, and low cost tool, which is also environmentally-friendly. Within the scope of this dissertation, we evaluated the potential of using freshwater fish as biological control tools, in its direct (predation itself) and indirect aspects (avoidance induction).

Initially, the potential of predation as a direct biological control method of *C. fluminea* was evaluated. As a first step, inspection of stomach contents of field-collected fish was performed in a network of canals where *C. fluminea* is highly abundant. The presence of this clam was only confirmed in *Barbus bocagei*'s stomachs, with a frequency of occurrence of 50%, but with a reduced relevance (< 5% stomach content). Considering these data and the available literature, two models were selected for predation experiments with *C. fluminea*: *B. bocagei* and *Lepomis gibbosus*. The interest shown by both fish species in *C. fluminea* individuals in predation experiments was extremely low, even with small-sized clams (< 1 cm). Additional experiments allowed demonstrating that the reduced utilization of *C. fluminea* as a prey was due to the protective role of its shell. In practical terms, the potential of these fish species as *C. fluminea* predators is reduced (*Barbus*) or null (*Lepomis*), precluding the possibility of using them as direct control tools.

Using *L. gibbosus* as experimental model in laboratory, the indirect effects of the presence of the fish on the behavior of *C. fluminea* were tested with avoidance experiments. These experiments relied on the prey's ability to modify its behavior when it perceives predator infochemicals. Results showed that the clams respond to the presence of the predator, by actively moving. However, the amplitude and direction of this dislocation depended on the presence of a refuge to the prey, the fish's diet and light conditions. This demonstrates the need to better understand these interactions, establishing the utility of predators (or its infochemicals) as a way to create exclusion zones in sensitive areas of water-dependent infrastructures.

Índice

INTRODUÇÃO GERAL

Introdução

A expansão dos humanos pelo mundo tem favorecido o aumento em grande escala da movimentação dos organismos de um ecossistema para outro. Com isto tem aumentado também o número de espécies exóticas que integram comunidades nativas, ameaçando ecossistemas, habitats e espécies (Oduor 1999).

Uma espécie ou subespécie não nativa, não indígena, exótica ou alienígena é aquela cuja distribuição se encontra fora dos seus limites naturais (Strayer 1999) passados ou presentes. Esta definição inclui qualquer parte, gâmeta ou propágulo destas espécies que possa sobreviver e, mais tarde, reproduzir-se (Cambray 2003b). A definição de espécie invasora estende-se a partir da de espécie não nativa, contemplando não só a introdução deliberada ou acidental de uma unidade taxonómica num local, mas também os fenómenos ecológicos associados à introdução e os impactos negativos que tal ação causa. Em 1996, peritos de 80 países concluíram que as espécies exóticas invasoras são a segunda maior ameaça à conservação da biodiversidade, logo a seguir à destruição de habitats (Neville 2002). A controvérsia gerada em torno dos termos associados às espécies introduzidas num novo local e seus efeitos associados veio exigir o estabelecimento de critérios objetivos para a sua classificação e caracterização, conforme o modelo sugerido por Colautti and MacIsaac (2004). Estes autores criaram um conjunto de termos a aplicar a estas espécies, com base em estágios do mecanismo de invasão, de modo a melhorar a clareza da nomenclatura usada em estudos futuros. Neste modelo, a cada estágio foi atribuído um número que corresponde a diferentes fases do possível processo de invasão (figura 1). Este modelo apresenta a vantagem de identificar os fatores que determinam o sucesso da invasão.

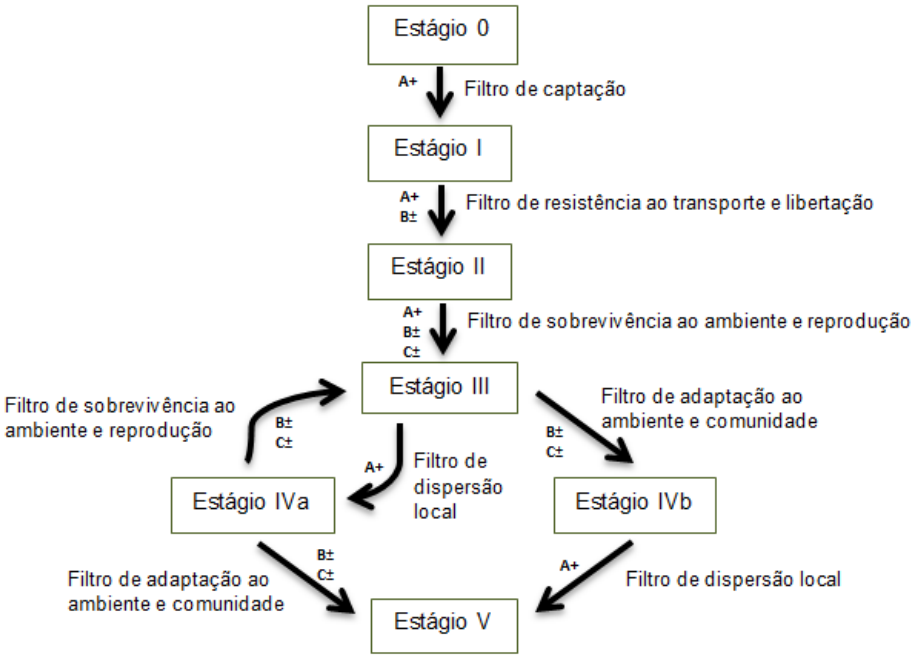

Figura 1 – Quadro sugerido para a aplicação operacional de termos numa invasão biológica – Adaptado de Colautti and MacIsaac (2004)

Segundo este modelo, a dinâmica da invasão biológica inicia-se no estágio 0, como um propágulo (Williamson and Fitter 1996) e, para que a espécie em causa seja considerada invasora, necessita de ultrapassar os filtros descritos entre cada estágio. Segundo este quadro, as espécies não-indígenas podem ter uma distribuição localizada e ser numericamente raras (estágio II), com larga distribuição mas raras (estágio IVa), ou com larga distribuição mas dominantes (estágio V). De acordo com estes autores, existem três possíveis determinantes que afetam a probabilidade do potencial invasor ultrapassar cada um destes filtros: pressão do propágulo (A), requisitos físico-químicos do potencial invasor (B) e interações na comunidade (C). Estes determinantes poderão afetar positiva (+) ou negativamente (-) o número de propágulos que ultrapassam os filtros. Neste modelo, uma espécie é considerada invasora se se encontrar nos estágios IVa ou V (Colautti and MacIsaac 2004).

Castri et al. (1990) enunciou as quatro principais etapas da invasão biológica, referindo-se à hipotética perda de potenciais invasores ao longo destas etapas: introdução (100 espécies), colonização (10 espécies), naturalização (5 espécies) e dispersão (2-3 espécies). A dispersão destas espécies poderá causar consideráveis impactos ecológicos,

sociais, económicos e genéticos e poderá funcionar como vetor para a transmissão de doenças, alterando o ecossistema e reduzindo a biodiversidade local (Lodge 1993, Cohen and Carlton 1998).

Impactos das espécies invasoras

As espécies invasoras são uma ameaça global à biodiversidade nativa provocando, em geral, modificações nos mais variados níveis de complexidade da área invadida e nos ecossistemas inerentes e afetando as condições abióticas locais - disponibilidade de luz, níveis de nutrientes e complexidade do habitat (Lodge 1993, Sousa et al. 2009). Estas espécies poderão modificar as caraterísticas do local invadido de diferentes formas, dependendo do(s) nível(is) de organização que afetam (Grosholz 2002).

Várias espécies têm sido descritas como invasoras com grandes impactos para os ecossistemas. Esta interferência a um nível tão elevado de organização biológica tem resultado em fortes alterações em sistemas de água doce e marinhos (Grosholz 2002, Sousa et al. 2009), afetando inclusivamente os serviços dos ecossistemas. Apesar da dificuldade associada à avaliação de impactos neste nível organizacional, vários autores abordaram os efeitos de espécies invasoras em níveis de organização biológica de complexidade elevada. Foi bem descrito o impacto de plantas invasoras nas comunidades e em serviços dos ecossistemas, apesar de não se conhecerem em detalhe as vias e mecanismos a ele associados. Ao estudar as ameaças destas plantas invasoras a este nível de complexidade é possível entender com mais pormenor a relação entre o invasor e as comunidades residentes. No estudo de Levine et al. (2003) foi descrito o impacto no ciclo de nutrientes, regime de fogo e hidrologia e alteração dos tempos de evapotranspiração. No Mediterrâneo, uma alga tem colonizado a costa desde Nice (França) até Imperia (Itália). Suspeita-se que este invasor, *Caulerpa taxifolia*, utilizado em aquários, tenha originado uma estirpe resistente à água fria, modificando drasticamente as comunidades costeiras, tanto ao nível funcional como estrutural. Esta alga pode modificar a qualidade e intensidade dos fatores físico-químicos locais, os abrigos para outros organismos, disponibilidade de alimento e a troca de indivíduos entre comunidades, alterando funcionalmente o ecossistema (BellanSantini et al. 1996, Bartoli and Boudouresque 1997, Thibaut and Meinesz 2004). Segundo Thibaut and

Meinesz (2004), dezoito anos depois da invasão por *C. taxifolia*, e com mais de 131 Km^2 afetados, os métodos de controlo físico-químicos e a consciencialização da população deixaram de ser uma solução viável, sendo agora a última opção o controlo biológico desta espécie.

Um outro exemplo relevante neste contexto é o da amêijoa invasora *Potamocorbula amurensis*, responsável por impactos ao nível das comunidades/ecossistema estuarino. A invasão do ecossistema nativo por esta espécie reduziu o fitoplâncton disponível, alterando a disponibilidade de recursos durante todo o ano. Esta produção primária natural sustentava grande parte do ecossistema pelágico da baía, que era dominada por zooplâncton e peixes. A pressão causada por esta amêijoa levou a uma reestruturação forçada do ecossistema da baía, mesmo que ainda se desconheça se é definitiva ou se foi resultado da combinação da presença do invasor e da condição hidrológica anómala dessa altura (Alpine and Cloern 1992, Cloern 1996, Grosholz 2002).

Algumas espécies invasoras mostraram afetar fortemente a distribuição de espécies nativas. Um desses casos é o mexilhão-zebra – *Dreissena polymorpha*. Este molusco de água doce, originário do Mar Cáspio e Rio Ural, Ásia, invadiu e colonizou a maior parte da Europa, a parte ocidental da Rússia, as zonas próximas da antiga União Soviética e a Turquia (Boelman et al. 1997). Crê-se que terá chegado aos Estados Unidos através de transporte em águas de balastro de navios transoceânicos (Molloy et al. 1997). Atuando como *biofouler*[1], rapidamente se estabeleceu em sistemas industriais, provocando problemas na captação de água de refrigeração e em embarcações, e causando prejuízos nas indústrias locais. Para além dos impactos económicos, o mexilhão zebra parece ter impactos significativos na ecologia das áreas invadidas (MacIsaac 1996) nomeadamente diminuindo a produtividade do fitoplâncton, incrementando a mortalidade dos bivalves nativos, e restruturando as comunidades bênticas (Molloy et al. 1997). No caso de duas trutas invasoras, *Salmo trutta* e *Oncorhynchus mykiss*, além de afetarem a distribuição da fauna

[1] *Biofouler* é o organismo que realiza *biofouling*, que consiste na degradação de superfícies artificiais subaquáticas como resultado de adesão, crescimento e reprodução destes organismos (Cao et al. 2011). Esta acumulação ocorre em superfícies como tubos, redes de pesca, tanques e cascos de navios, resultado em corrosão, entupimento, contaminação ou diminuição da eficiência das estruturas (Yebra et al. 2004, Cao et al. 2011).

piscícola nativa, afetaram também as populações de lagostim (Townsend 2003), anfíbios e macroinvertebrados presentes na África do Sul, através da predação, competição por habitat e por alimento (Cambray 2003b, Cambray 2003a).

Também a amêijoa asiática, *Corbicula fluminea*, tem sido responsável por impactos ao nível das populações. Em 2001 foi testado o impacto desta espécie na fauna bentónica presente em Goose Creek, Estados Unidos, tanto *in situ* como em laboratório. A presença de *Corbicula fluminea* neste local mostrou impactos significativos nas populações de bactérias e flagelados (Hakenkamp et al. 2001). Em Portugal, esta espécie também demonstrou ser capaz de causar impactos negativos na fauna nativa (incluindo os bivalves) de vários rios, tais como Minho (Sousa et al. 2008), Lima (Sousa et al. 2006), Mondego (Chainho et al. 2006), Guadiana (Morais et al. 2009), Sado (Morais et al. 2009) e Tejo (Mouthon 1981). É particularmente preocupante a associação entre o seu sucesso (invasão) e o declínio dos bivalves nativos (Unionidae e Margaritiferidae), quer na Europa (Sousa et al. 2008), quer nos Estados Unidos (Hakenkamp et al. 2001).

Vários estudos têm quantificado o impacto das espécies de moluscos invasores na demografia de determinadas espécies nativas (Araujo et al. 1993, Rius and McQuaid 2006). Através de modelos demográficos, é possível determinar as consequências associadas ao estabelecimento de uma determinada espécie invasora, ao nível da sobrevivência, do crescimento e da reprodução da espécie nativa em estudo (Townsend 2003). No entanto, os impactos a níveis de organização inferiores podem demorar muito tempo até se refletir em níveis de organização superior, podendo ser difícil de quantificar ou de prever impactos à macroescala com base nestes efeitos (Cambray 2003b). Por exemplo, nas Galápagos, um estudo com a duração de 7 anos demonstrou que a presença do invasor *Cinchona pubescens* causou impactos negativos inicialmente numa espécie de planta herbácea endémica, mas também mais tarde ao nível da comunidade (Jager et al. 2007, Jager et al. 2009). Também outra espécie de planta endémica, na Califórnia, *Oenothera deltoides* ssp. *howellii*, está a sofrer as consequências da invasão de outra planta, *Bromus diandrus*, que impede o estabelecimento das plântulas da espécie nativa (Thomson 2005). Em última análise, a presença de um invasor poderá conduzir à redução da densidade de uma espécie nativa numa comunidade local (Jager et al. 2009) ou até à sua extinção (Mooney and Cleland 2001), o que é particularmente gravoso no caso de espécies endémicas, como no caso do endemismo ibérico *Unio tumidiformis* (Reis and Araujo 2009) sujeito ao invasor *Corbicula fluminea*.

Os impactos ecológicos apresentados nos níveis superiores de organização biológica terão efeitos significativos na composição do biota de uma região e na paisagem, contribuindo drasticamente para o declínio da biodiversidade e alterando a estrutura e o funcionamento dos ecossistemas. Com a previsão da crescente globalização dos mercados mundiais, exploração e fragmentação dos ecossistemas naturais e turismo, e com a movimentação de um número progressivamente mais elevado de pessoas para os ambientes ainda não explorados, tende a ocorrer um aumento dramático da dispersão das espécies não nativas, tanto local como globalmente. De um modo drástico, mas em proporções reais, e como refere Marchante et al. (2003), "está assim a caminhar-se para a criação de um mundo uniforme, onde faltará a identidade local de cada habitat".

Além dos impactos no ambiente, estas espécies poderão afetar as indústrias e economia humana que dependam direta ou indiretamente dos recursos naturais e dos elementos biológicos nativos. Isto conduzirá obrigatoriamente a maiores custos para o Homem, tanto na monitorização e no controlo de invasores, como em soluções para as consequências ecológicas ou económicas que eles determinam. Estas soluções passam, por exemplo, pelo controlo físico, químico ou biológico (temas abordados mais à frente neste documento) e pelo investimento em estratégias de monitorização de espécies invasoras e locais invadidos (Higgins et al. 2000, Olson and Roy 2002). A dimensão económica do problema das espécies invasoras pode ilustrar-se facilmente com os estragos causados nos habitats naturais promovidos pela invasão por cerca de 50 000 espécies de animais e plantas ocorrida até 1980 nos Estados Unidos, que se traduziram em custos estimados em 120 105 milhões de dólares.

Características das espécies invasoras

Uma espécie invasora com larga distribuição nativa estará adaptada a um leque mais variado de condições ambientais e, portanto, terá maior probabilidade de sucesso num novo local colonizado do que uma espécie de distribuição nativa e tolerâncias ambientais mais limitadas. Na verdade, mais de metade das espécies invasoras apresenta um comportamento invasor em mais do que uma região (Reichard and Hamilton 1997). Sabe-se que as espécies invasoras demonstram geralmente algumas características comuns, tais

como: ocupar uma grande área nativa, larga abundância, alimentação generalista, tempo de geração curto, alta variabilidade genética, comportamento gregário, um rápido crescimento, maturidade precoce e amplo intervalo de tolerâncias fisiológicas (Lodge 1993, Hokkanen and Lynch 1995, McMahon 2002).

Vários autores têm relacionado esta amplitude na gama de tolerância das espécies invasoras a habitats variados, entre estes os habitats extremos, com o sucesso do processo de invasão (Lodge 1993, Hokkanen and Lynch 1995, McMahon 2002). Karatayev et al. (2007) referiu e comparou as características, tolerâncias abióticas, dispersão e impactos nos ecossistemas de dois moluscos invasores, *Dreissena polymorpha* e *Limnoperna fortunei* (mexilhão-dourado). O autor concluiu que estas espécies tinham características comuns: ambos sésseis, com uma fase larval planctónica e alta capacidade reprodutiva. Ambos os adultos atingem uma biomassa muito superior ao das espécies de invertebrados nativos nas massas de água afetadas. Neste estudo, o autor refere que *Limnoperna fortunei* apresenta tolerâncias fisiológicas mais alargadas e que, apesar de os impactos de *Dreissena polymorpha* serem mais conhecidos e, por isso, ser uma espécie mais bem estudada, o mexilhão-dourado apresenta potencial para ser um invasor ainda mais agressivo. McMahon (2002) testou também a relação entre as elevadas tolerâncias e o potencial invasor em espécies aquáticas na América do Norte, particularmente em *Dreissena polymorpha* – mexilhão-zebra - e *Corbicula fluminea* – amêijoa asiática. Estas duas espécies invasoras apresentam caraterísticas das espécies do tipo *r* – adaptadas a condições instáveis, com pouca competição interespecífica. A população nativa de bivalves na área de estudo apresentava caraterísticas contrárias à da população exótica – espécie do tipo *K*: baixa fecundidade, habitam locais raramente perturbados, com densidades que tendem a atingir a capacidade de carga do ambiente, resultando numa competição inter e intraespecífica pelos limitados recursos. No entanto, estas últimas evoluíram no sentido de evitar a extinção, desenvolvendo mecanismos de sobrevivência quando expostas a situações extremas. Assim sendo, este estudo acabou por concluir que tanto as espécies invasoras como as nativas poderiam apresentar tolerâncias fisiológicas semelhantes, sugerindo que as gamas alargadas de tolerância fisiológica a variações ambientais não será uma caraterística exclusiva de espécies invasoras.

A adaptação a diferentes habitats e as elevadas tolerâncias fisiológicas das espécies invasoras têm sido alvo de estudo também na área da genética. Lee (2002) demonstrou que os mecanismos de invasão são determinados geneticamente. Segundo o autor, o sucesso

da invasão é condicionado pela arquitetura genética dentro das populações de origem e a seleção natural nesse substrato genético leva a adaptações rápidas em novos ambientes. Mais especificamente, o autor concluiu que um pequeno número de genes poderá ter elevados efeitos sobre o sucesso da invasão.

O presente trabalho de dissertação focou-se no bivalve invasor *Corbicula fluminea*, comummente designado de amêijoa asiática. Trata-se de um invasor geralmente bem-sucedido nos ecossistemas onde é introduzido (Britton 1982, Huybregts and Tamburri 2005, Sousa et al. 2008), o que naturalmente está relacionado com as suas caraterísticas biológicas específicas (Sousa et al. 2008).

***Corbicula fluminea* como invasor bem-sucedido**

A amêijoa asiática é um dos bivalves com maior capacidade invasora (Werner and Rothhaupt 2007), tanto considerando medidas de abundância como de biomassa (Pérez-Quintero 2008). Originária do Sudeste Asiático, coloniza preferencialmente ecossistemas de água doce e suspeita-se que terá sido introduzida deliberadamente na costa oeste dos Estados Unidos por volta de 1930 (Phelps 1994). Depois disso há evidências que terá percorrido os Estados Unidos através de transporte passivo, sobretudo associado a atividades humanas (embarcações de pesca ou recreativas, uso enquanto isco de pesca) mas também a aves marinhas (Britton 1982), à semelhança de *Dreissena polymorpha*, cujas larvas e juvenis foram transportados pelo pato-real (Bilton et al. 2001). Um dos primeiros registos de ocorrência de *C. fluminea* fora da sua área nativa data de 1924, na América do Norte (Beran 2006), enquanto na Europa foi registado pela primeira vez em 1980, em França - Basse Dordogne – e em Portugal, no Estuário do Tejo (Mouthon 1981). A partir de então têm sido relatados vários registos de *Corbicula fluminea* em Portugal: no rio Douro (Nagel 1989), no rio Lima (Sousa et al. 2006), no rio Mondego (Chainho et al. 2006), no rio Minho (Sousa et al. 2008), no rio Sado e Guadiana (Morais et al. 2009), na barragem de Santa Clara, em Mira, e barragem da Bravura, no Algarve (Rosa et al. 2011). Atualmente, esta espécie está disseminada um pouco por toda a Europa e América, encontrando-se ainda em dispersão e estando classificada como um dos cem piores agentes biológicos invasores (DAISIE 2008).

A espécie *Corbicula fluminea* pertence ao filo Mollusca, um dos maiores do grupo dos animais invertebrados – integra cerca de 120 000 espécies (Oliveira 1987). A maioria dos organismos deste filo são marinhos, mas também congrega espécies de água doce e espécies terrestres (Álvarez-Fernández and Carvajal-Contreras 2008). A classe Bivalvia, pertencente ao filo Mollusca, engloba cerca de 25 000 espécies (Oliveira 1987) e carateriza-se pela presença de uma concha constituída por duas valvas comprimidas lateralmente. Esta concha é segregada pelo manto e este abriga na sua cavidade os ctenídeos - órgãos responsáveis pela respiração -, o ânus, a extremidade dos nefrídeos – responsáveis pelos produtos de excreção - e os quimiorrecetores (Yeh 2003, Pechenik 2005). No caso da amêijoa asiática, a forma do pé evoluiu vantajosamente, sendo utilizado para o animal se enterrar no sedimento, o que acontece na presença de predadores, e para apoiar a locomoção. Esta espécie pertence à superfamília Corbiculoidea (inclui as famílias Corbiculidae e Sphaeriidae), que agrupa espécies que apresentam uma concha aproximadamente triangular – figura 2 – e que se reproduzem de forma ovovivípara. A fertilização ocorre internamente e os ctenídeos possuem modificações que lhes permitem servir como câmara de incubação de ovos e larvas; os novos recrutas são libertados já completamente formados (com concha), quais cópias miniaturizadas dos seus progenitores (Britton and Fuller 1980, King et al. 1986, Kraemer and Galloway 1986).

Figura 2 – *Corbicula fluminea*

Os indivíduos da espécie *Corbicula fluminea* podem alimentar-se de diferentes formas: filtração, parcialmente seletiva, que é dominante no ecossistema invadido (Boltovskoy et al. 1995, Hakenkamp and Palmer 1999, Strayer et al. 1999); *pedal-feeding*,

em que o pé é usado para revolver o sedimento e provocar a ressuspensão de partículas orgânicas, que serão posteriormente filtradas (Reid et al. 1992, Hakenkamp and Palmer 1999, Hakenkamp et al. 2001).

Este invasor coloniza os mais variados habitats: rios, albufeiras, lagos e zonas salobras. Sousa et al. (2008) referem o aparecimento desta espécie em todos os tipos de sedimentos. Encontrada em zonas com fortes correntes, é uma espécie tolerante à variação de diversas condições ambientais (ver os exemplos fornecidos na tabela 1), à exceção do oxigénio (McMahon 1979, Britton 1982).

Tabela 1 – Limites abióticos registados na literatura para *Corbicula fluminea* (Karatayev et al. 2005).

FATOR ABIÓTICO	LIMITES DE TOLERÂNCIA	
	Inferior	**Superior**
Salinidade (‰)	2	10-17
Temperatura (ºC)	2	36-37
pH	5,6	Não definido

Além da elevada tolerância a variações nas condições abióticas, *Corbicula fluminea* apresenta outras caraterísticas que lhe conferem uma grande capacidade invasora. Segundo McMahon (2002), esta espécie apresenta elevada fecundidade - 35000 descendentes por indivíduo adulto - e Doherty et al. (1987) referem a existência de três períodos de libertação de juvenis: um no início da primavera, um no meio do verão e outro no início do outono. No entanto, o número de eventos reprodutivos poderá estar relacionado com a temperatura da água (Rajagopal et al. 2000, Mouthon 2001a) e com os recursos alimentares disponíveis no ecossistema (Cataldo and Boltovskoy 1998, Mouthon 2001b, a). Sendo uma espécie hermafrodita em que a fecundação cruzada não é obrigatória, um único indivíduo de *Corbicula fluminea* poderá dar continuidade à espécie. Apesar disso, é mais comum a fertilização cruzada (Mouthon 1981).

Vários estudos têm registado impactos industriais e económicos relacionados com a invasão de *Corbicula fluminea*, principalmente em indústrias dependentes de água doce em que esta espécie atua como um *biofouler* (Doherty et al. 1987, Rosa et al. 2011). Rosa et al. (2011) enunciaram os principais tipos de indústrias dependentes de água doce existentes em

Portugal que estariam propensas à invasão ou já com problemas associados. Neste estudo, os autores referiram a existência de impactos moderados nestas indústrias, sendo particularmente relevantes neste contexto os setores da irrigação e do tratamento de águas para consumo humano.

O *biofouling* em sistemas dependentes de água causado por esta espécie é um problema grave em indústrias, podendo inclusivamente comprometer a segurança dos equipamentos. Alguns dos problemas que as indústrias dependentes de água enfrentam com esta espécie são: bloqueio de canos/tubagens, menor eficácia nos sistemas de arrefecimento da água e corrosão acrescida (Johnson et al. 1986). Em indústrias de produção de energia nuclear, são frequentemente afetados os sistemas de segurança contra incêndio, as bombas de injeção de emergência e os sistemas de refrigeração de reatores nucleares (Johnson et al. 1986). Muitas vezes, a infestação exige ainda a remoção periódica das populações instaladas e eventual reparação de material e sistemas, o que naturalmente representa custos acrescidos. Só nos Estados Unidos, estima-se que os danos causados por *Corbicula fluminea* e a implementação de técnicas de controlo tenham custado 1000 milhões de dólares por volta de 1980 (OTA 1993, Pimentel et al. 2005). Em Portugal, os custos estimam-se em 200 000€ anuais (Rosa et al. 2011). Em particular no caso de alguns bivalves, para mexilhão-zebra (*Dreissena polymorpha*) estes custos rondam os $1000 milhões, assim como para *Corbicula fluminea* (Pimentel et al. 2005). Nos *Great Lakes*, América do Norte, as autoridades estimam que em 2012, o controlo associado ao mexilhão-zebra (*Dreissena polymorpha*) em indústrias de abastecimento de água municipais custará mais de $5 mil milhões durante dez anos, com utilização de métodos físicos e químicos (NOAA 2012).

Potenciais métodos de controlo de invasores

Considerando os impactos e custos associados às espécies invasoras, e as dificuldades em prever os efeitos que uma nova introdução causará, a melhor solução seria simplesmente impedir as introduções de espécies exóticas. Apesar de insuficiente para evitar problemas, e por já ser demasiado tarde (Taylor and Hastings 2004), a legislação que regula as movimentações de espécies exóticas é muito importante (Bean 1999). Além disto,

a prevenção de introduções envolve menos custos do que o controlo do invasor, depois de estabelecido (Westbrooks and Eplee 1999, Mehta et al. 2007). Através de modelos populacionais, a dispersão dos invasores tem sido registada, auxiliando com sucesso a compreensão das dinâmicas das invasões biológicas (Higgins and Richardson 1996, Sousa et al. 2006). Não obstante a permanente vigilância para prevenir novas invasões, permanece a necessidade de encontrar ferramentas de controlo para as espécies invasoras existentes. Em alguns casos, como o da amêijoa invasora *C. fluminea* e do mexilhão invasor *D. polymorpha*, existe a necessidade de métodos de controlo eficazes para fazer face aos impactos que produzem na indústria (*biofouling*).

O controlo de invasores pode apresentar-se de três formas: mecânico, químico e biológico (Melchior 1997). No controlo químico de bivalves, utilizam-se frequentemente compostos com ação biocida (Cloete et al. 1998). Os biocidas mais utilizados são substâncias oxidantes como o cloro, dióxido de cloro, cloraminas, ozono, bromo, peróxido de hidrogénio, permanganato de potássio, entre outros. O uso destes biocidas é particularmente preocupante pelos riscos que coloca aos técnicos que os aplicam, pelos impactos em organismos não-alvo, e pela corrosão que poderá provocar em superfícies metálicas e ligações elétricas.

Dadas as desvantagens destes agentes altamente oxidantes, têm sido desenvolvidas outras alternativas, como por exemplo as toxinas produzidas pela bactéria *Pseudomonas fluorescens* (estirpe CL145A), comercializadas sobre o nome Zequanox^{TM} (Marrone Bio Innovations, Inc.). Esta formulação está em fase de testes para o controlo de *Dreissena* spp. (Mackie and Claudi 2010). Um outro desenvolvimento tecnológico é a encapsulação de biocidas (e.g. KCl) em microcápsulas (e.g. BioBullets; ver Aldridge et al. (2006)), o que permite uma administração direta e seletiva do agente biocida nos organismos-alvo, que retêm as microcápsulas que, mais tarde, libertam o agente biocida. Este é um método particularmente indicado para bivalves (Mackie and Claudi 2010), que, pela sua elevada capacidade filtradora, se tornam alvos destas cápsulas feitas à medida. A seletividade do método permite reduzir a quantidade de agente biocida a usar, já que este é direcionado de forma muito direta para o alvo numa forma concentrada, e minimizar fenómenos de evitamento por parte dos organismos-alvo (por exemplo, *D. polymorpha* fecha as valvas ao detetar a presença de cloro na água – ver Aldridge et al. (2006)). Vários estudos têm demonstrado que os métodos de controlo químico são indicados essencialmente para populações controladas ou isoladas, tais como indústrias hidrodependentes, onde é feito o

devido tratamento às águas residuais antes de serem lançadas para o ambiente (Mackie and Claudi 2010). Os possíveis efeitos nefastos em organismos não-alvo desaconselham o uso de métodos químicos de controlo diretamente no ambiente (Sharov and Liebhold 1998, Olson and Roy 2002, NOAA 2012).

Alguns dos métodos de controlo físico utilizados são a água aquecida (McMahon and Ussery 1995), indicada para equipamentos de campo e embarcações ou para motores e tubagens se aplicada em contra-corrente. Outros métodos físicos incluem a dessecação, que requer mais tempo de aplicação, de acordo com a resistência do bivalve, e o esmagamento, como medida eficaz de remoção de bivalves de maior tamanho e em áreas pequenas (NOAA 2012).

Frequentemente, as indústrias afetadas não apresentam equipamentos que possam reduzir o estabelecimento do invasor. Por exemplo, é recomendável que locais passíveis de invasão possam ser isolados para remoção de organismos ou desinfeção da área, de forma regular (USACE 2011). A prevenção é uma estratégia menos onerosa do que o controlo, mas nem sempre poderá ser aplicada. Nos casos em que o controlo continua a ser necessário, os métodos de controlo biológico podem ser uma estratégia eficiente, com menos custos ambientais, e com sucesso no longo-termo.

Quando aplicado no meio natural, o controlo biológico pode envolver riscos (Hokkanen and Lynch 1995, Sandlund 1999). Numerosas introduções de indivíduos para controlo biológico têm afetado organismos não-alvo por insuficiência de preocupação com os potenciais problemas que o agente biológico poderia causar (Simberloff and Stiling 1996). Por exemplo, a presença de um novo predador (agente de controlo) na região afetada poderá causar um declínio de uma espécie nativa. Um exemplo desta situação foi a introdução dos peixes *Gambusia affinis* e *G. holbrooki* em várias regiões para controlar mosquitos, que causou a extinção local de algumas espécies nativas de peixes (Lloyd 1990). Efetivamente, o agente biológico introduzido para controlo irá estabelecer interações competitivas e tróficas com a população nativa, podendo alterar o ecossistema. Outro problema associado ao controlo biológico é a dificuldade de quantificar o valor das espécies, tanto intrínseca como economicamente e, por isso, a análise custo/benefícios é dificilmente calculável (Simberloff and Stiling 1996, Follett and Duan 2000). Também nesse sentido, é preciso considerar a ética relativa ao uso de uma espécie como ferramenta de controlo biológico; mais, é necessário ponderar se a espécie introduzida para fins de controlo de outra dever ser retirada ou se necessita permanecer no local (Simberloff and Stiling 1996).

Ainda assim, o controlo biológico deve ser um método de controlo com a finalidade de resolver problemas de invasão biológica com a mínima perturbação nas comunidades nativas. O maior objetivo dos métodos deste tipo é a redução e estabilização a longo termo da densidade populacional da espécie-alvo, garantindo o balanço entre a população invasora e os seus inimigos naturais, de modo que nenhum dos dois se torne demasiado abundante, assim assegurando a biodiversidade natural local (Oduor 1999).

Os organismos vivos que são inimigos naturais de espécies invasoras podem ser classificados como parasitas, parasitóides, patogéneos, predadores (frequentemente utilizados como métodos diretos de controlo), antagonistas ou competidores (utilizados como métodos indiretos). A diferença entre parasitas e parasitóides consiste no efeito que induzem no hospedeiro, que em ambos os casos é favorável ao uso no controlo da espécie afetada – os parasitas enfraquecem ou reduzem o sucesso reprodutivo dos hospedeiros e os parasitóides desenvolvem-se no interior do organismo-alvo, matando-o posteriormente. Os patogéneos são geralmente micro-organismos, sendo que os hospedeiros mortos libertam posteriormente à sua morte milhões de micróbios que serão transmitidos entre hospedeiros (Oduor 1999). O uso de predadores tem sido particularmente explorado porque cada predador consome um largo número de presas naturalmente durante a sua vida. Os antagonistas e competidores são potenciais ferramentas para evitar o estabelecimento de um invasor num local particular de invasão. Todos estes inimigos naturais têm a capacidade de manter os invasores em baixos níveis de abundância, assim controlando as suas populações e impedindo que dominem o sistema (Oduor 1999). Nesta dissertação, serão explorados a predação e o antagonismo, este último na forma de evitamento, como potenciais medidas de controlo direto e indireto (respetivamente) do bivalve invasor *Corbicula fluminea*.

A predação, enquanto medida de controlo biológico, tem demonstrado algum sucesso, particularmente em experiências com recurso a insetos (Caltagirone 1989, Dixon 2000, Nomikou et al. 2001, Bellows et al. 2006). Em 2004 foi testado o potencial da predação como medida de controlo biológico para *biofouling* nas conchas de organismos (vieira, *Pecten maximus*) e redes de aquaculturas (Ross et al. 2004). Os autores demonstraram que uma espécie de cavalo-marinho e uma de caranguejo eremita eram potenciais ferramentas de controlo biológico, eliminando um sério problema de *fouling*. Estas espécies reduziram o *biofouling* em 50% e também os custos associados à limpeza das embarcações e redes de pesca anteriormente afetadas, com a vantagem das espécies predadoras serem comuns e

disponíveis. A aplicação da predação no controlo biológico de moluscos também tem demonstrado resultados, sobretudo quando associados a problemas de biofouling (Dumont et al. 2009), mas também quando associados a perturbações em ecossistemas (Oliveira et al. 2010).

A sobrevivência das presas depende da sua habilidade de reconhecer os predadores e das suas adaptações para os evitar. Em ambiente aquático, as pistas químicas (infoquímicos ou semioquímicos) permitem de forma fidedigna que a presa reconheça e evite os riscos associados a um predador (Stoecker 1980, Greenstone and Dickens 2005). Apesar de ainda não estar devidamente esclarecida a natureza dos infoquímicos libertados por predadores (cairomonas; Dicke and Sabelis 1988, Burks and Lodge 2002), existem evidências de que a inclusão prévia da espécie-presa na dieta pode contribuir para a força do sinal (Stabell et al. 2003). Em experiências de evitamento, é possível testar se a presa responde de forma inata a cairomonas ou se apresenta uma aprendizagem individual ou social desses sinais (Ferrari et al. 2005). Entre estes sinais, os alarmes químicos têm sido estudados numa grande variedade de organismos, incluindo protozoários, platelmintes, anelídeos, artrópodes, moluscos, peixes e anfíbios. Em moluscos, foi testado o efeito de cairomonas de potenciais predadores em *D. polymorpha*, tendo-se registado um maior índice de agregação da presa na presença de predadores (Kobak and Kakareko 2009). A indução de respostas comportamentais anti-predador por parte da presa apresenta potencial, por isso, para ser usada como mecanismo indireto de controlo biológico (Nelson et al. 2004, Ramirez et al. 2010).

Objetivos e estrutura da dissertação

Os problemas ecológicos e económicos previamente enunciados resultantes da presença de *Corbicula fluminea* têm sido alvo de preocupações. Neste contexto, a presente dissertação tinha como objetivo explorar o potencial de métodos de controlo biológico direto e indireto desta espécie invasora. No capítulo 1, como uma primeira abordagem, pretendeu-se avaliar quais os potenciais predadores desta espécie e a possível aplicação da predação como medida de controlo direto, através de experiências utilizando diferentes espécies de peixes potencialmente predadoras de *C. fluminea*. Também neste capítulo se explorou o

efeito protetor da concha desta espécie como agente impeditivo da predação. Mais adiante, no contexto do capítulo 2, analisaram-se os efeitos provocados pela presença do predador no comportamento do invasor, com especial ênfase nos comportamentos de fuga/evitamento. Os dados gerados na segunda parte da dissertação constituem uma primeira abordagem à possibilidade da utilização de zonas de exclusão de espécies invasoras, potenciadas pela presença de predadores, como potenciais métodos de controlo indireto das mesmas. No último capítulo desta dissertação (considerações finais), tecem-se algumas considerações sobre a relevância dos resultados obtidos e o potencial dos diferentes tipos de métodos biológicos explorados no controlo biológico de espécies invasoras e em particular de bivalves invasores como a amêijoa asiática.

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CHAPTER 1

DIRECT METHODS FOR BIOLOGICAL CONTROL – PREDATION

1. INTRODUCTION

Invasive species are a global threat to the native biodiversity and cause ecological damages that may potentially alter the structure and function of the receiver ecosystems (Mooney and Cleland 2001), as well as social, economic and genetic impacts. This leads not only to biodiversity management concerns but also to costs in monitoring and/or controlling the invaders and finding solutions to their environmental and economic consequences (Lodge 1993, Cohen and Carlton 1998).

Finding an effective solution to control or eradicate invasive species depends on several variables, including the scientific knowledge available about the species and its interaction with the recipient environment and native biota. Although there is not a defined standard method, there are several techniques available for controlling invaders in isolated areas, such as affected water-dependent industries (Sharov and Liebhold 1998, Olson and Roy 2002, Mackie and Claudi 2010). These methods can be chemical, physical, mechanical or biological (Melchior 1997), and their use is strongly dependent of a cost-benefit analysis. Some techniques, such as heat treatments or mechanical removal, can be costly and/or slow acting. Chemical treatment, like chlorination, has been the most commonly used method to eradicate invasive species for the last 50 years in both Europe and United States of America (Pimentel et al. 2005), particularly because it generally provides higher cost efficiency; however, it has drawbacks, such as the likely discharge of hazardous materials into the environment (Pimentel 2005). Low cost efficient solutions that concomitantly are eco-friendly are still unlikely to exist; hence, the quest for improved control methods is worth continuing.

In this way, biological control is an eco-friendly alternative to control invasive species that can be applied by direct methods, using parasites, parasitoids, pathogens or predators. Indirect methods are also possible, using antagonists or competitors (Oduor 1999). Due to the accelerated invasion rates registered nowadays around the world, the control potential of predation has been increasingly investigated, being considered by some the most ecological solution to eliminate invaders (Driesche et al. 2008). However, it is a control technique with some associated risks to the native ecosystem. This method may affect non-target native species or, if it is not carefully followed or monitored, it may result on the introduction of other nuisances (Howarth 1991, Boettner et al. 2000, Henneman and Memmott 2001). The major advantage of biological control by predation is that the use of natural enemies/predators does

not generally involve additional costs or management in the long term. Natural predators are self-sustaining, self-dispersing and usually adjust their population's size to their prey's population (Messing and Wright 2006).

In what concerns the control of invasive freshwater bivalves, which can become serious nuisances in water-dependent facilities, several methods have been suggested (see Mackie and Claudi 2010). In some cases, a combination of intrinsically distinct methods showed to be a safest and more successful alternative to eradicate invasive bivalves such as the Zebra and Quagga mussels (McMahon and Ussery 1995, WRP 2009). In the Great Lakes, the authorities estimated that the control of zebra mussel (*Dreissena polymorpha*) in municipal water intake facilities will cost more than 4 billion € during ten years, using physical and chemical methods (NOAA 2012). No successful examples of biological control are known with the most notorious invasive freshwater bivalves (*D. polymorpha*, *Corbicula fluminea*).

The Asian clam, *Corbicula fluminea*, is an example of a successful invasive bivalve (Werner and Rothhaupt 2007, Pérez-Quintero 2008), and there are suspicions that its introduction in some areas was deliberate (Phelps 1994). It colonizes preferably freshwater systems but also brackish waters. *Corbicula fluminea* is tolerant to most abiotic parameters except to oxygen level (McMahon 1979, Britton 1982). Its original distribution was confined to Asia, Africa and Australia in the beginning of the last century (Mouthon 1981). Nowadays, it can be found with a large distribution in the US and Europe, particularly in France and Portugal, where it has already colonized a high number of freshwater systems (Mouthon 1981, Britton 1982, Araujo et al. 1993, Reis 2006, Rosa et al. 2011). The Asian clam colonization capacity is believed to be facilitated, as in most invasive species, by the inefficiency of natural competitors and predators (Oduor 1999). For example, this species was reported in Minho river (Portugal) in 1989 and, after a short time it became dominant relatively to the native benthic fauna in terms of abundance and biomass (Sousa et al. 2008). In locations where native competitors or predators constrain the dispersal and establishment of invasive species, their potential as efficient tools for biological control needs to be studied (Oduor 1999, Ross et al. 2004).

Some fish have been reported to consume mollusks (McMahon et al. 1977), in particular invasive bivalves (Cloe et al. 1995, Lévêque 1995, Agostinho et al. 2009, Oliveira et al. 2010). To be effective predators of bivalves, such as *C. fluminea*, moluscivorous fish require morphological pre-adaptations in order to crush the shell and subsequently digest the soft tissues (French 1993, Darrigran and Damborenea 2006, Oliveira et al. 2010). Garcia &

Montalto (2006) noticed that fish that effectively predate *C. fluminea* in the Upper Paraná River (e.g. *Pterodoras granulosus*) generally had mouths adapted for suction or were provided with strong incisiform or molariform teeth, and in some cases pharyngeal teeth. French (1993) recognized pharyngeal teeth and chewing pads as essential features of a moluscivore. This is the case of several cyprinid species (e.g. *Barbus* sp., *Cyprinus carpio*) and pumpkinseed (*Lepomis gibbosus*) (French 1993), fish species that have been confirmed as predators of non-native *C. fluminea* or *Dreissena polimorpha* in field situations in Europe and in the US (Boles and Fellow 1993, Kelleher et al. 2000).

In this work, we evaluate predation as a potential method of biological control of the clam *C. fluminea.* We focused on a network of canals in Mira, whose semi-lotic characteristics are morphologically and ecologically representative of the irrigation canals, and drinking water supply facilities usually affected by this pest. Fish and clams coexist and, in some areas, clams are extremely abundant (densities from 2000 to 4000 individuals/m^2; Inês Rosa and Joana Pereira, personal communication). Three specific objectives were pursued: 1) to identify potential predators in the area, by conducting gut content analysis of local fish and reviewing available literature; 2) to assess size-dependent predation, experimentally, in order to address the most effective predator size and its impacts in the clam's size structure; 3) to evaluate the preventing role of the clam's shell as an obstacle to predation.

2. METHODOLOGY AND MATERIALS

2.1 STUDY SITE AND ANIMALS

Corbicula fluminea individuals were collected from a canal network in Mira, Portugal, in November. Clams were collected using a shovel to drag sediment into a porous bag (1 mm mesh size), which was then used to roughly sieve the sample and separate clams along with larger debris. The sieved sample was then sorted out for selection of clams with shell length ranging from < 5 to 25 mm. The selected clams were immediately transported to the laboratory in 20 L buckets partially filled with local water (ca. 500 individuals per bucket). In the laboratory, clams were separated by size class and progressively transferred to dechlorinated tap water, where they were maintained under continuous aeration and weekly

water renewal. Clams were fed *ad libitum* with green microalgae suspensions (*Pseudokirchneriella subcapitata*) after every renewal.

Fish were immobilized and caught with a portable electrofishing gear. Depending on the use (experiments or guts content analysis), fish were either stored on ice *in situ* or transported in aerated containers filled with local water. Only the most abundant and non-endangered species were caught to minimize impacts to the native ecosystem. They were collected in the same canal network to ensure the cohabitation with the Asian clam, their potential prey.

2.2 ASSESSMENT OF POTENTIAL FISH PREDATORS

Fish collected at the Mira canal system were dissected in the laboratory to analyze their stomach contents. The whole digestive tract was analyzed under a stereoscope for the presence of *C. fluminea* shell fragments. We calculated the frequency of occurrence (FO) (Hyslop 1980) of *Corbicula fluminea* as a prey:

FO = 100 x stomachs with *Corbicula fluminea* / total analyzed stomachs.

Based on stomach content analysis (see results) and bibliographic data, we selected *Barbus bocagei* and *Lepomis gibbosus* as model predators for the experiments.

2.3 SIZE-DEPENDENT PREDATION EXPERIMENTS

Field-collected *B. bocagei* were separated by size class: small (S, <30 cm), intermediate (I, 30-40 cm) and large fish (L, > 40 cm). The smallest individual had 21 cm and the largest 57 cm. Twelve high-density polyethylene conical containers (outdoor enclosures, 1000 L) with 1.2 m and 1.0 m diameter at top and bottom, respectively (Pereira et al. 2010), were used in the experiments. Enclosures were displayed in a 4x3 arrangement at the same distance from each other in CRAM - Rehabilitation Centre of Marine Animals of Quiaios (an infrastructure of the Portuguese Wildlife Society, SPVS). The twelve recipients were filled with 400 L of tap water and aerated for 24 h prior to the experiments. Fish were placed in the enclosures and allowed an acclimation period of 24 h, without food. Aeration was kept constant throughout the experiment. To initiate the experience, fish (predator) were distributed according to size class (3 size classes x 3 replicate enclosures): 1 fish per enclosure in size class L, 2 fish per enclosure in size class I, and 4 fish per enclosure in size

class S. This guaranteed an approximately similar biomass of fish among size classes. The remaining 3 enclosures were utilized as negative controls, where no fish were added. To avoid the interference of abiotic factors or other bias, treatments were randomly distributed. All enclosures were covered with a plastic net (5 mm mesh size) to prevent entrance of animals other than the ones we experimentally manipulated. The feeding experiment started when 125 clams (prey) were introduced in each enclosure, including controls. Each group of clams in an enclosure was constituted by 25 individuals of 5 size classes: 5-10, 10-12.5, 12.5-15, 15-17.5, and 17.5-20 mm. During the experiment, predators were allowed to feed on *C. fluminea* for 24 h. After this period, clams were recovered, counted and measured to quantify the proportion of ingested prey in each size class.

A similar experiment was performed with *Lepomis gibbosus*, but in laboratory aquaria. Field-collected individuals were separated by size and placed in 10 L aquaria with dechlorinated tap water. Aquaria were aerated for 24 h prior to the experiments, and fish were not fed. The feeding experiment began with the addition of 40 clams per aquarium, equally distributed by 4 class sizes: 5-10, 10-15, 15-20 and 20-25 mm. Unlike for *B. bocagei*, only large pumpkinseed (>10 cm) were used in the experiments, because smaller specimens are gape-limited. Therefore, only two treatments were carried out in this experience – one with one fish and the other as a negative control, without fish – using three replicate aquaria each. Aeration was kept constant throughout the experiment. At the end of a 24 h period, the clams were removed, counted and measured, to quantify the proportion of ingested prey in each size class.

Basic water quality parameters were registered at the beginning and at the end of the experiment using a multiparameter probe, for quality assurance criteria: pH, dissolved oxygen (% saturation), conductivity (µS/cm) and temperature (ºC).

2.4 PREVENTIVE ROLE OF THE CLAM'S SHELL IN PREDATION

In order to assess the role of the hard shell of *C. fluminea* in preventing predation, we conducted experiments where fish were offered both open and closed (live) clams. This also allowed us to verify the potential nutritional value of the prey and the degree of interest of the predator in the prey.

Experiments with *B. bocagei* followed the same rationale and procedure than in the size-dependent predation experiment, except in the way clams (prey) were added. We used a total of 720 clams, by introducing 60 per enclosure, half of which were forced open to expose the soft tissues, immediately before adding them to the enclosures; the other half was supplied alive with their shell closed. Each group of 30 clams (either open or closed) was equally distributed by 3 class sizes: 10-15, 15-20 and 20-25 mm. We reduced clam density and size classes relatively to the first experiment to minimize the number of clams experiencing mechanical sacrifice. The feeding experiment lasted for 24 h. After this period, clams were recovered, counted and measured to quantify the proportion of ingested prey in each size class. They were also frozen for posterior estimation of soft body biomass, by extracting individual soft tissues and drying (60ºC) them to constant weight.

For the experiment with *L. gibbosus*, we followed the exact same design of the size-dependent predation experiment, except that half of the 40 clams were supplied opened, equally distributed along the 4 class sizes used in the former experiment with this fish species.

At the end of the experiments, all fish were released in the Mira canal system, where they were originally caught.

2.5 STATISTICAL ANALYSIS

Data on the proportion of *Corbicula* eaten in the various experiments were analyzed for differences among experimental treatments with the Kruskal-Wallis test. The use of such a non-parametric procedure was necessary because of the binomial nature of the data and its non-normality and heteroscedasticity.

3. RESULTS

3.1 ASSESSMENT OF POTENTIAL FISH PREDATORS

Seven fish species were caught in the Mira canal system: *B. bocagei*, *L. gibbosus* (non-native), *Cobitis* sp., *Cyprinus carpio* (non-native), *Liza aurata*, *Anguilla anguilla*, and *Micropterus salmoides* (non-native). This included medium-sized and large fish, whose gape size theoretically allowed ingestion of clams up to 2 cm. Of these, cyprinids *B. bocagei* and *C. carpio*, as well as the centrarchid *L. gibbosus*, were documented as potential moluscivores, given their morphological adaptations and feeding habits (see introduction). However, we found *C. fluminea* shell fragments only in *B. bocagei* stomachs (FO = 50%). Even in these individuals, shell fragments represented a very low proportion of total stomach contents (< 5% in volume).

3.2 SIZE-DEPENDENT PREDATION EXPERIMENTS

Barbus bocagei

Predation experiments with *Barbus bocagei* revealed that clams were eaten only by intermediate and large fish (table 1). However, no statistically significant differences were found among treatments (Kruskal-Wallis test: H = 5.23, d.f. = 3 and P = 0.156). This is likely to be due to the very low proportion of *Corbicula* eaten and to inconsistencies among replicates within each treatment (table 1). The average proportion eaten was 2.40 ± 2.88 % (intermediate fish) and 5.07 ± 8.09 % (large fish). The maximum number of *Corbicula* eaten was recorded in enclosure A, where a large barbel (TL = 46 cm) ate 18 clams out of 125.

Table 1 – Initial and final conditions of size-dependent predation experiments for *Barbus bocagei*

Treatment		Replicate	Number of *Corbicula*			Proportion eaten (%)
			introduced	recovered	eaten	
No fish		J	125	125	0	0
		K	125	125	0	0
		L	125	125	0	0
Fish	Small	B	125	125	0	0
		E	125	125	0	0
		I	125	125	0	0
	Interm	D	125	118	7	5.6
		G	125	123	2	1.6
		H	125	125	0	0
	Large	A	125	107	18	14.4
		C	125	124	1	0.8
		F	125	125	0	0

Figure 1 shows the size class distribution of eaten *Corbicula* in enclosures A (large fish, n = 18) and D (intermediate fish, n = 6). Although the low number of clams eaten constrains a consistent analysis of the size preferences of the predator, it is clear that fish were able to eat clams from 0.5 cm up to 2 cm (figure 1), suggesting there is no gape-limitation for these size classes.

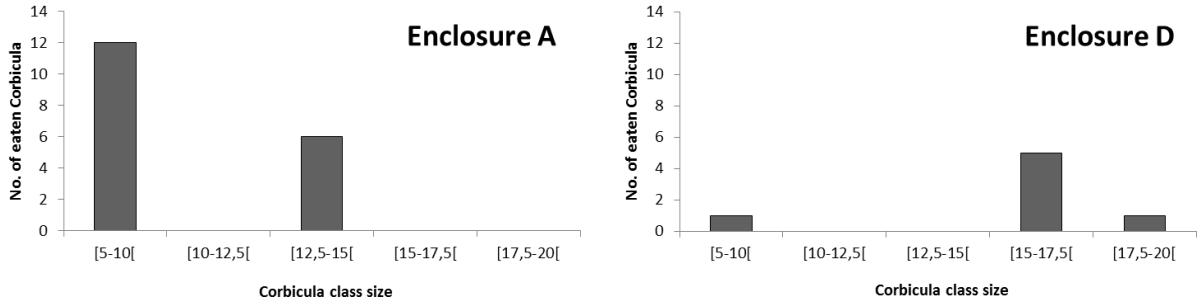

Figure 1 – Number of eaten clams per size class in enclosures A (large fish) and D (intermediate fish)

Lepomis gibbosus

In the *L. gibbosus* experiments, all *Corbicula* were recovered, thus producing a consistent outcome of no fish predation (table 2). Concurringly, no statistically significant differences were found between fish treatments (Kruskal-Wallis test: H = 0.00, d.f. = 1 and *P* = 1.000)

Table 2 – Initial and final conditions of size dependent predation experiments for *Lepomis gibbosus*

Treatment	Replicate	Number of *Corbicula*			Proportion eaten (%)
		introduced	recovered	eaten	
No fish	A	40	40	0	0
	B	40	40	0	0
	C	40	40	0	0
Fish	D	40	40	0	0
	E	40	40	0	0
	F	40	40	0	0

3.3 PREVENTIVE ROLE OF THE CLAM'S SHELL IN PREDATION

Barbus bocagei

Experiments testing the preventive role of the clams' shell in predation by *Barbus bocagei* revealed that only small fish ate *Corbicula* and these clearly preferred open clams (table 3); none of the closed clams was predated in these experiments. Small fish consumed a significantly higher proportion of open clams (63.33 ± 15.28 %) than intermediate or large fish (0 %) (Kruskal-Wallis test: H = 10.73, d.f. = 3 and *P* = 0.013). Open clams were consumed in all enclosures with small fish (table 3). In these enclosures, small fish apparently exhibited a preference for larger (open) clams (figure 2). The maximum number of *Corbicula* eaten was recorded in enclosure G, where the small barbels ate 24 out of the 30 open clams provided.

Table 3 –Initial and final conditions of the predation experiment where both open (o) and closed (c) clams were made available to *Barbus bocagei*.

Treatment		Replicate	Number of *Corbicula*			Proportion eaten (%)
			introduced	recovered	eaten	
No fish		J	30 (o) + 30 (c)	30 (o) + 30 (c)	0 (o) + 0 (c)	0 (o) + 0 (c)
		K	30 (o) + 30 (c)	30 (o) + 30 (c)	0 (o) + 0 (c)	0 (o) + 0 (c)
		L	30 (o) + 30 (c)	30 (o) + 30 (c)	0 (o) + 0 (c)	0 (o) + 0 (c)
Fish	Small	D	30 (o) + 30 (c)	15 (o) + 30 (c)	15 (o) + 0 (c)	50 (o) + 0 (c)
		F	30 (o) + 30 (c)	12 (o) + 30 (c)	18 (o) + 0 (c)	60 (o) + 0 (c)
		G	30 (o) + 30 (c)	6 (o) + 30 (c)	24 (o) + 0 (c)	80 (o) + 0 (c)
	Interm	B	30 (o) + 30 (c)	30 (o) + 30 (c)	0 (o) + 0 (c)	0 (o) + 0 (c)
		E	30 (o) + 30 (c)	30 (o) + 30 (c)	0 (o) + 0 (c)	0 (o) + 0 (c)
		I	30 (o) + 30 (c)	30 (o) + 30 (c)	0 (o) + 0 (c)	0 (o) + 0 (c)
	Large	A	30 (o) + 30 (c)	30 (o) + 30 (c)	0 (o) + 0 (c)	0 (o) + 0 (c)
		C	30 (o) + 30 (c)	30 (o) + 30 (c)	0 (o) + 0 (c)	0 (o) + 0 (c)
		H	30 (o) + 30 (c)	30 (o) + 30 (c)	0 (o) + 0 (c)	0 (o) + 0 (c)

In order to confirm that the classification eaten/not eaten was unambiguous, we determined the average weight of the soft tissues present in the open clams at the end of the experiment. Results shown for the small fish treatment (figure 3) – which was the only one where open clams were preyed upon – demonstrate that nearly all soft tissues were removed from the shell when predation occurred. In fact, visual inspection revealed that only fragments of adductor muscles and mantle remained attached to the shell of open clams that had been scored as eaten. These results support the objectivity of the visual difference between eaten and not eaten *Corbicula*, and sustain the unbiased estimation of the proportion of open *Corbicula* eaten.

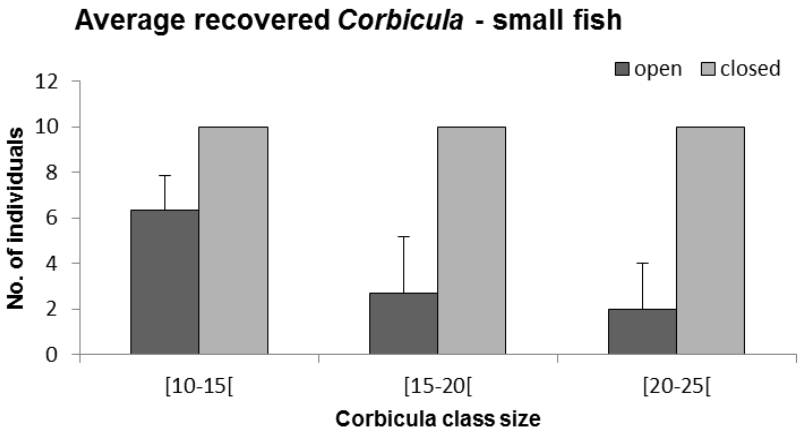

Figure 2 – Average number of clams recovered in small fish enclosures and their distribution by the three class sizes. Error bars represent standard-deviation.

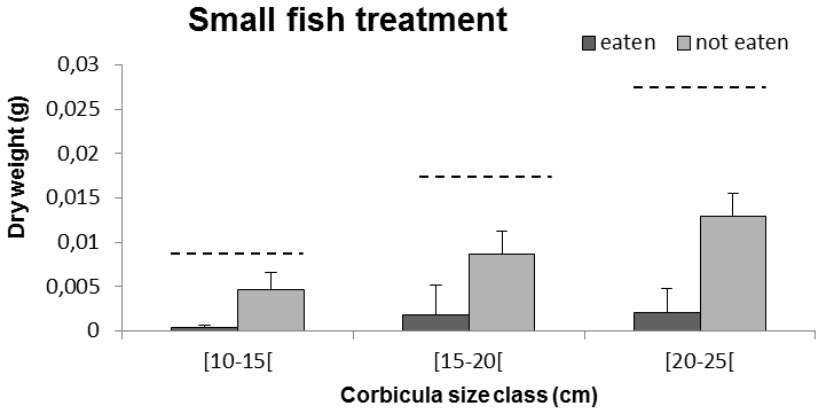

Figure 3 - Average biomass (dry weight, g) of soft tissues from open clams visually scored as eaten or not eaten in the small fish treatment. Dashed lines represent the average biomass in the negative control for each prey size class. Error bars represent standard-deviation.

Lepomis gibbosus

Almost all open *Corbicula* were eaten by *L. gibbosus* (table 4), which translated into a statistically significant effect of treatment (fish vs. no fish) on the predation of open clams by this fish species (Kruskal-Wallis test: H = 4.50, d.f. = 1 and *P* = 0.034). As observed in *Barbus bocagei* experiments, none of the closed clams were eaten.

Table 4 – Initial and final conditions of the experiment assessing the preventive role of the shell as an obstacle for predation by *Lepomis gibbosus*

Treatment	Replicate	Number of *Corbicula*			Proportion eaten (%)
		introduced	recovered	eaten	
No fish	A	20 (o) + 20 (c)	20 (o) + 20 (c)	0 (o) + 0 (c)	0 (o) + 0 (c)
	B	20 (o) + 20 (c)	20 (o) + 20 (c)	0 (o) + 0 (c)	0 (o) + 0 (c)
	C	20 (o) + 20 (c)	20 (o) + 20 (c)	0 (o) + 0 (c)	0 (o) + 0 (c)
Fish	D	20 (o) + 20 (c)	0 (o) + 20 (c)	20 (o) + 0 (c)	100 (o) + 0 (c)
	E	20 (o) + 20 (c)	8 (o) + 20 (c)	12 (o) + 0 (c)	60 (o) + 0 (c)
	F	20 (o) + 20 (c)	0 (o) + 20 (c)	20 (o) + 0 (c)	100 (o) + 0 (c)

4. DISCUSSION

The approach here described intended to evaluate the potential of several fish species as natural predators of *C. fluminea*, in sites where this clam is invasive. Ultimately, we intended to assess their potential as biological control methods of the pest. However, the results obtained were characterized by some degree of inconsistency and reduced evidence of fish foraging on clams, suggesting predation of live *C. fluminea* by fish is not frequent. Taking into account that *C. fluminea* is an extremely abundant resource in the study site, we must conclude that one of its secrets as a successful invader also resides on their low susceptibility to fish predators, including the clam's smaller cohorts (as shown here).

Other authors have shown that fish predation may be a useful tool in integrated pest management programs of some bivalves, despite the invaders' successful characteristics (Robinson and Wellborn 1988, McCrady 1991, Magoulick and Lewis 2002, Oliveira et al. 2010). Robinson and Wellborn (1988) concluded that fish predation had a twenty-nine fold negative effect on the abundance of *Corbicula fluminea* in a Texas reservoir. This study proved a large magnitude effect of fish in abundance of the Asian clam, when compared to

some previous studies in other macroinvertebrates (Thorp and Bergey 1981, Flecker and Allan 1984, Gilinsky 1984, Reice and Edwards 1986). In a study with the zebra mussel (*D. polymorpha*), Magoulick and Lewis (2002) demonstrated that native fish predators in Lake Dardanelle (Arkansas, US) can decrease adult *D. polymorpha* colonization, but are unlikely to limit the mussels' population density because of the prey's reproductive potential.

Only *Barbus bocagei* was found to prey on *C. fluminea* in the Mira channel system; however, the low importance of prey ingested (FO = 50%, % volume < 5%) was indicative of the low interest of the fish in this potential and abundant food resource. Similarly, Oliveira et al. (2010) analyzed the importance of two invasive bivalves, *Corbicula fluminea* and *Limnoperma fortunei*, in the diet of some fish, and found that 12 out of 36 fish consumed *Corbicula fluminea* but this clam did not comprise more than 10% of their diet. Gut content analysis does not allow discriminating between accidental and voluntary ingestion. Clams could already be dead, intact or fragmented and the fish, along with its opportunistic feeding attempts (Collares-Pereira et al. 1996), could have eaten the shells accidently (Collares-Pereira et al. 1996, Gaspar da Luz et al. 2002). This causes additional uncertainty in the predatory potential of *B. bocagei*. Due to the high fragmentation of the shells found in the stomachs of *Barbus bocagei* collected in the field, we could not determine the size of the ingested clams. However, the size of clams may be important to define the strategies for biological control. Depending on the most predated size class, management may be directed to suppressing the sexually mature individuals (sexual maturity - 6.5 mm shell size according to Aldridge and Mcmahon (1978)) or juveniles. To properly address the uncertainty relatively to the potential of barbels as predators of *C. fluminea*, we carried out manipulation experiments, which we will discuss later.

No signs of predation were found in the other analyzed fish, although their characteristics are favorable to predate bivalves (e.g. *Lepomis gibbosus*, *Cyprinus carpio*; French 1993). Despite of being referred in bibliography as moluscivorous fishes (Collar and Wainwright 2009), none of the *Lepomis gibbosus* or *Cyprinus carpio* individuals ate *Corbicula fluminea*. Despite the large abundances of *Corbicula fluminea* available, the mechanical costs to crush the Asian clam may be the cause of the absence of predation. This was confirmed in experiments when *L. gibbosus* were fed with live *C. fluminea*. This species feeds on zooplankton (mainly while juveniles) and dipterans (Hanson and Qadri 1984, Garcia-Berthou and Moreno-Amich 2000, Castro and Goncalves 2011); snails are only consumed when fish are > 35 mm length, and even with this size they may not have developed the pharyngeal

teeth, which depend on the repeated, consistent consumption of snails (Bailey 1938 cited in Collar and Wainwright 2009). Freshwater snails, because of their dimensions and typical morphology, are not comparable as a prey to bivalves, whose hard shell may be a strong deterrent to predation. Indeed, when *L. gibbosus* was fed with open (dead) clams, it consistently foraged on the clam's soft tissues while closed (live) clams were left untouched. This is a strong indication that the shell is an evident deterrent of potential predators. Moreover, this suggests that, when *C. fluminea* experience mortality due to external factors (viruses, oxygen depletion, other unknown causes), dead clams may become important resources for local fish, in parallel to what Sousa et al. (2012) suggested for aquatic-terrestrial coupling when massive die-offs occur.

In size-dependent experiments, only the larger *B. bocagei* ate *C. fluminea* and in very small amounts. However, this was not statistically significant, given the very low amount of ingested clams, and the inconsistency among replicates. However, other studies show the importance of the predator size in a moluscivore diet (Collares-Pereira et al. 1996). For example, roach (*Rutilus rutilus*) changes its diet from algae and macrophytes to Zebra mussels, when it reaches 16 cm length, in Lake Sniardwy. When they reach about 28 cm, their diet is mostly moluscivore (Prejs et al. 1990). In our experiments, smaller fish did not eat live *Corbicula*, which could be explained by their gape limitation (Collares-Pereira et al. 1996) or by an extreme expense of crushing, even for the smaller clams (Prejs et al. 1990). Indeed, as discussed for *L. gibbosus* (see above), the clams' shell seems to be a serious obstacle to predation, even in fish equipped to crush mollusk shells. A priori, we expected that larger fish would consume larger prey and smaller fish would consume at least the smaller clams, as observed in other moluscivorous fish's experiments (McClintock and Robnett 1986, Prejs et al. 1990, Ray and Corkum 1997). Overall, our results reveal a negligible effect of *B. bocagei*'s as a predator of the Asian clam; indeed, its potential as a biological control tool seems very limited. One cannot exclude that the stress of capture and confinement could have increased stress levels and/or diminished the fish's appetite during the experiments; however, while experiments were not being conducted, we occasionally fed them with frozen chironomid larvae (Frozen Fish Food; Schijndel, The Netherlands), which they ate after a few hours (*Barbus bocagei* is described to eat dipteran larvae (Magalhães 1992)). Thus, fish seem to be active and feeding, even though in captivity.

Similarly to *L. gibbosus*, we tested the protective role of *C. fluminea*'s shell on *B. bocagei*, as the shells may be conditioning predation (Cantanhêde et al. 2008, Oliveira et al.

2010), whether in size or in thickness. For example, Stein et al. (1975) found that, in the moluscivorous *Cyprinus carpio*, the obstacle to predation of mollusks was mainly the thickness of the shells. In a study on predation of mollusks by some crustaceans, the authors found that crabs and lobsters prefer small-sized preys, which can be explained by the mechanical predation cost (energy spent on breaking the shell) to the predator – Optimal Foraging Theory: if predation costs are part of a fixed budget and there is a limited resource, then the probability of failure must be considered as a possible additional cost parameter. (Macarthur and Pianka 1966, Stephens and Krebs 1986, Prejs et al. 1990, Juanes 1992). In our experiments, only small fish ate *Corbicula*, at a significantly higher proportion than intermediate and large fish. Although these results partly support the idea that the clams' soft tissues are interesting to barbels as prey, the results are nevertheless contradictory. Larger barbels did not consume neither closed nor open clams, although they had preyed upon *C. fluminea* in the size-preference experiments. At this point, we must assume that larger fish could be experiencing some stress, as fish were confined progressively more time out of their natural environment and were manipulated for the second time, to re-distribute them for the enclosures. While this was apparently not true for smaller fish, larger fish require larger volumes (Harvey and Stewart 1991) and, thus, are putatively more susceptible to stress due to confinement. Probably due to this, results from this experiment were less conclusive than the ones with *L. gibbosus*, regarding the protective role of the Asian clam shell.

In conclusion, both fish species revealed low potential as predators of *C. fluminea*, mostly due to the efficient antipredator defense provided by the hard shell of this invasive clam. Despite the evidences found in the stomach content of *Barbus bocagei* and the bibliographic references suggesting its suitability as a natural predator, our experiments revealed some incipiency as to the potential of predation by this fish species as an efficient tool for the biological control of the Asian clam. Still, larger-scale experiments with *B. bocagei* should be carried out, eventually considering longer time periods, to fully characterize the predatory activity of the species over the Asian clam and allow more feasible conclusions on its applicability as a biological control method. On the contrary, our experiments indicated the unsuitability of *Lepomis gibbosus* as a biological control agent for the Asian clam through predation, although its molluscivory has been suggested in the literature. It would be of great interest to further investigate the protective strategies of the Asian clam against predation by the tested fish. Also, further research on the efficiency of alternative vertebrate (e.g. the freshwater catfish) and invertebrate predators (e.g. the freshwater crayfish) should constitute

a meaningful follow-up to this study, in order to shed some light on the possibility of using predation to control invasive bivalves.

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CHAPTER 2

INDIRECT METHODS FOR BIOLOGICAL CONTROL OF CORBICULA FLUMINEA – AVOIDANCE EXPERIMENTS USING PREDATORS

1. INTRODUCTION

Invasive species are a global threat to the native biodiversity and may alter the structure and function of the colonized ecosystems (Mooney and Cleland 2001), but also the local abiotic conditions such as light availability, nutrient levels and habitat complexity (Sousa et al. 2009). Besides the ecological impacts, these species can also affect human activities and consequently the economy that depends directly or indirectly on the natural resources or on the native biological elements that they disturb (Higgins et al. 2000, Olson and Roy 2002). There is not an ecologically- or economically-ideal method defined as a model solution to control invasive species, but there are many tools available that have been tested and proven efficient to control some invaders in isolated areas such as water-dependent facilities (Sharov and Liebhold 1998, Olson and Roy 2002). These tools can be chemical, physical, mechanical or biological (Melchior 1997), and their use is strongly dependent on a cost-benefit analysis. Low cost but efficient solutions, that concomitantly are eco-friendly, are still scarce, thus the quest for improved control methods is still ongoing.

Living organisms that are natural enemies of invasive species can be classified as parasites, parasitoids, pathogens, predators, antagonists or competitors. They can be used as biological control agents, profiting from direct or indirect effects on the nuisance species. These organisms potentially avoid the establishment of an invasive species in a particular area, keeping invaders at low abundance levels and preventing their dominance (Oduor 1999). Therefore, the survivorship of the invaders, as that of the rest of potential preys, depends on their ability to recognize the predators and on their adaptations to avoid them. In aquatic organisms, this avoidance behavior relies mostly on chemical cues (infochemicals or semiochemicals) that allow the prey to acknowledge predation risk (Stoecker 1980, Greenstone and Dickens 2005). The chemical cues that constitute inter-specific signatures used by the preys to detect the risk are named kairomones (Dicke and Sabelis 1988, Dodson et al. 1994, Kats and Dill 1998, Grostal and Dicke 1999, Burks and Lodge 2002, Wisenden 2003). There is no consensus about the nature of kairomones released by predators, but it has been suggested that increased signal strength occurs when the prey has been part of the predator's diet or when body damage is caused by predators (Stabell et al. 2003, Wisenden 2003, Ferrari et al. 2005). The response to kairomones has been experimentally assessed through avoidance experiments, which may provide important information for the

development of biological methods to control invasive species (El-Sayed et al. 2006, Meurisse et al. 2008). For example, Kobak and Kakareko (2009) noticed that predator kairomones elicited enhanced bivalve aggregation in the sessile stages of the invader *Dreissena polymorpha* – zebra mussel. In more mobile organisms, the use of predators or predator cues could allow implementing exclusion zones in critical areas of water-dependent facilities, by profiting of the anti-predator avoidance behavior of the invader. Hence, in areas where local native competitors or predators are known to constrain the dispersal and establishment of invasive species, detailed scrutiny on the involved interactions can provide crucial information on their potential as efficient tools for biological control (Oduor 1999, Ross et al. 2004).

The Asian clam, *Corbicula fluminea*, is another example of a successful invasive bivalve (Werner and Rothhaupt 2007, Pérez-Quintero 2008), and several studies have reported industrial and economic impacts related with infestation by this species (e.g. Rosa et al. 2011). Unlike the zebra mussel, it has some mobility, which makes it a suitable case study for the use of its antipredator avoidance behaviour as an indirect control method. Its original distribution was confined to Asia, Africa and Australia in the beginning of the last century (Mouthon 1981) but nowadays it can be found largely distributed in the US and in Europe, particularly in France and Portugal, where it has already colonized a high number of freshwater systems (Mouthon 1981, Britton 1982, Araujo et al. 1993, Reis 2006, Rosa et al. 2011). The successful progression and establishment of this clam has been suggested to be mostly grounded on several characteristics of its life-cycle (e.g., short life span, high fecundity (Doherty et al. 1987, McMahon 2002) but also to be related to its wide-range tolerance to different environmental parameters (McMahon 1979, Britton 1982). Also, some researchers argue that the successful colonization capacity of the invasive species is facilitated by the relative inefficiency of natural competitors and predators (e.g. Oduor 1999). Indeed, invasive species are known for their higher effectiveness in avoiding predators as compared to native species (Weis 2011). For example, the rusty crayfish *Orconectes rusticus* demonstrated more effective anti-predator behavior (decreased activity and swimming) against the largemouth bass *Micropterus salmoides* than the native crayfish *Orconectes virilis* (Garvey et al. 1994).

The present study was aimed at testing predator-induced avoidance as a potential indirect method of biological control applicable to the Asian clam *C. fluminea*. To do this, coexistence experiments were carried out with the clam and one of its potential predators, the pumpkinseed sunfish *Lepomis gibbosus* (Boles and Fellow 1993, French 1993, Molloy et al.

1997), and the escape behavior of the prey was assessed. Essentially, we hypothesize that the clams should perceive the presence of the predator by detecting released kairomones, thus avoiding predation risk by moving towards an accessible predator-free area. Experimental evidence on the suitability of a predator-induced clam-free area would be a breakthrough for the development of efficient control methods to apply in/around critical underwater structures. Different conditions that could influence the behavioral response of the clams were considered in the experimental design, including: (a) fish diet (presence/absence of clams in the diet); (b) the availability of a refuge; (c) the illumination (presence/absence of light).

2. METHODOLOGY AND MATERIALS

2.1 Animals collection and maintenance

Corbicula fluminea individuals were collected from a canal network in Mira, Portugal, in November. Clams were collected using a shovel to drag sediment into a porous bag (1 mm mesh size), which was then used to roughly sieve the sample and separate clams along with larger debris. The clams were immediately transported to the laboratory in 20 L buckets partially filled with local water (ca. 500 individuals per bucket). In the laboratory, clams were progressively transferred to dechlorinated tap water, where they were maintained under continuous aeration and weekly water renewal. Clams were fed *ad libitum* with green microalgae suspensions (*Pseudokirchneriella subcapitata*) after every renewal.

Fish (*Lepomis gibbosus*) were immobilized and caught with a portable electrofishing gear and transported in aerated containers filled with local water. They were collected in the same canal network to ensure the cohabitation with the Asian clam, their potential prey. Large specimens (> 10 cm) were chosen for the experiments and were kept in 10 L aquaria with dechlorinated tap water. *L. gibbosus* is particularly suited for experimentation in the laboratory because of its size, ease of manipulation, requirements and absence of a protection status (it is a nuisance alien species) (Rodrigues et al. 2011).

To assess the effect of fish diet in the antipredator avoidance response of *C. fluminea*, two groups of fish were used: six fish were fed opened *C. fluminea* (valves forced open

immediately before placing them in the aquaria), and the other 6 were fed with frozen chironomid larvae (Frozen Fish Food; Schijndel, The Netherlands), three times a week. This guaranteed a group of predators with previous contact with the prey and a group of predators without recent contact with *C. fluminea*.

2.2 Development of the test system

In order to test the effect of the presence of a predator on the escape behavior of *C. fluminea*, a controlled test system was progressively improved. The first test system consisted in two 5-L plastic buckets (22Hx17Ø cm) connected by a semi-transparent tube (5 mm diameter) (figure 1a); this tube was intended to hold the clams while allowing their movement through a putative kairomone gradient. Both buckets were filled with dechlorinated tap water and a fish (*Lepomis gibbosus*) was transferred to one of them. Two clams were placed in the middle section of the tube and their movement (towards or opposing the predator) was recorded. The rounded inner surface of the tube was found to be limiting, because two clams could not be placed side-by-side at the same relative distance from the buckets; thus, the movement of one clam could constrain the movement of another regardless the influence of the chemical signal.

A second test system was then developed by placing the two buckets in opposite sides of a 60-80-L glass aquarium (80Lx30Wx40H cm). The bottom of the buckets was removed and sealed with a net (0.5 cm mesh size). The buckets were suspended ca. 3 cm above the bottom of the aquarium, and one of them was used to retain one fish (*L. gibbosus*) (figure 1b). Ten clams were then placed under each bucket and their movements were recorded hourly, using a digital camera. A major limitation of this system was the difficulty in assessing the movements of each individual, as well as their direction. Also, the system did not allow isolating avoidance behavior and other responses to predator signals, such as aggregation, which could constitute an undesirable interference.

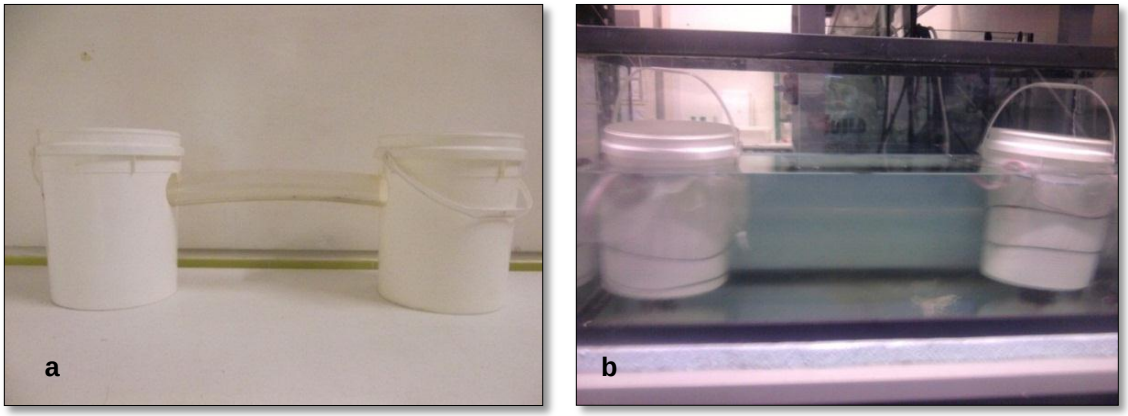


Figure 1 – First (a) and second (b) preliminary experimental systems tested for assessing *C. fluminea*'s avoidance from a potential predator signal.

The development of a third system (see figure 2) allowed limiting the clam's movement in two directions: towards the fish or in the opposite direction. We divided test aquaria (50Lx25Wx30H cm) in two halves with a glass plate, used as a divider. This allowed isolating the fish predator in one half of the aquarium. The bottom of each aquarium was equipped with 6 plastic rails (ca. 40 x 2 x 1 cm; L x W x H) that constituted runways for the clams to move; each rail held one clam. The clams were placed in the runways, in the midline of the aquarium, aligned with the divider (see figure 2a). Their movement was recorded with a digital camera (see below).

2.3 Definitive experimental design and procedures

A bifactorial design was used taking into consideration the fish diet ("fish factor") and the potentially protective effect of isolating the clams from direct contact with the predator ("net factor"). The presence of the net (extended throughout the bottom of the aquarium – see figure 2) prevented physical contact between predator and prey, thus allowing us to distinguish avoidance phenomena strictly associated to the kairomone stimulus from avoidance from physical contact (e.g. fish poking the clams). Three replicates were carried out for each combination of fish and net treatments (see figure 4). A negative control (i.e. no fish) was also carried out in triplicate and treated as a level of the fish factor (figure 3). Thus, the fish factor comprised 3 levels: (a) negative control with no fish predator added; (b) fish

predator previously fed with *C. fluminea*, placed in one half of the aquaria; (c) fish predator previously fed with chironomid larvae, placed in one half of the aquaria. This experimental design was applied both under permanent illumination (24 h light) and under permanent darkness (24 h dark), to assess the effect of light in the avoidance response.

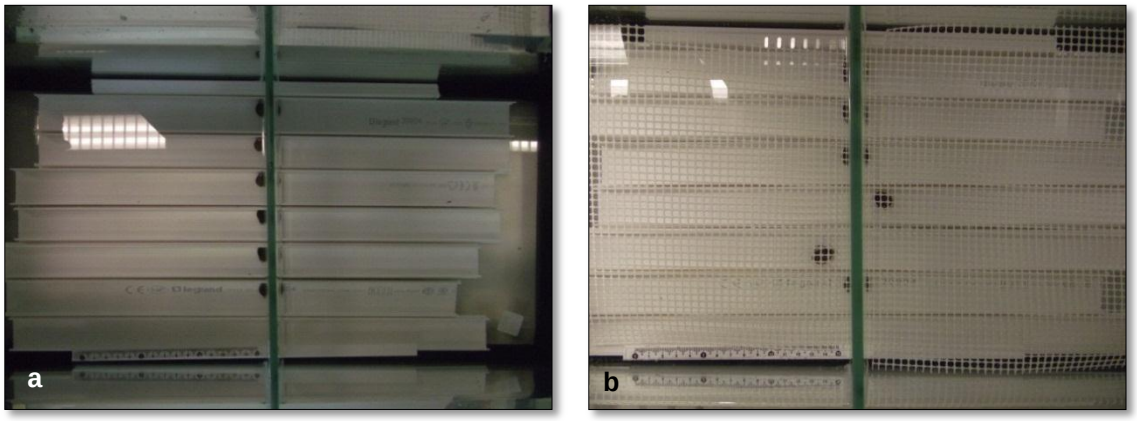
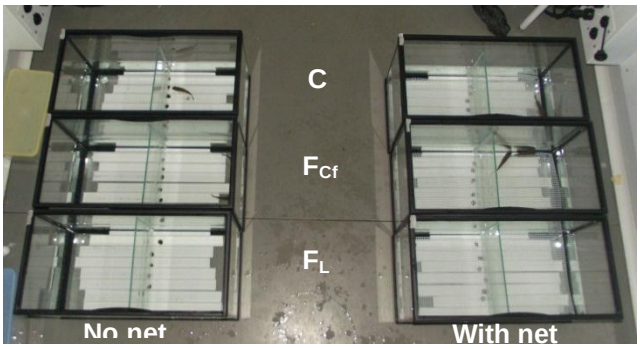


Figure 2 – Examples of two test conditions established in the definitive test system, before adding the predator. (a) Aquarium with no protective net; (b) Aquarium with protective net. Notice the ruler (bottom left), which was used as a reference to measure the distance travelled by each individual, relatively to the middle line (glass plate divider).



C – Control
F_{Cf} – Fish fed *Corbicula fluminea*
F_L – Fish fed larvae

Figure 3 – Initial setting of the experimental design, showing the two levels of the net factor (net; no net) crossed with the three levels of the fish factor: (C) control with no fish predator added; (F_{Cf}) fish predator previously fed with *Corbicula fluminea*; (F_L) fish predator previously fed with larva.

The aquaria were half-filled with dechlorinated tap water and six clams (one per corridor) were used as the experimental subjects. Given the anatomical position of the clam's foot, it is likely that the movement of a clam depends on its initial spatial orientation. To minimize such bias, all clams were placed standing on their left valve, with three facing the fish-free area (posterior end facing the fish) three facing the fish (anterior end facing the fish) (figure 4).

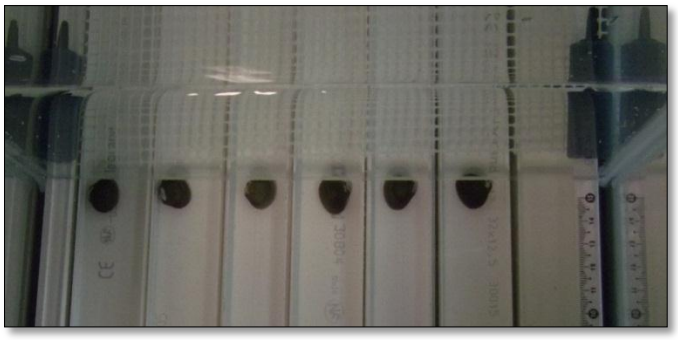


Figure 4 – Clams' initial disposal in the rails.

Based on earlier assessments, the experimental period was set to 24 h – preliminary observations showed clam's activity and specifically movement towards a single direction

mainly in the first 24 h. Indeed, the size and configuration of the system suggests that any kairomone gradient that establishes should become uniform within hours, which could constrain the assessment of the avoidance behavior. The position of the clams in the aquarium was recorded photographically at the 2nd, 4th, 6th and 24th hour of the experimental period (see figure 5). Images were analyzed using Image J software (Abramoff et al. 2004). The photographs were calibrated using the scale attached to the rail, and the distance travelled by each clam relatively to the middle line (glass plate divider) was recorded (cm). Displacement towards or away from the predator were distinguished as negative (preference) or positive (avoidance; escape) distances, respectively.

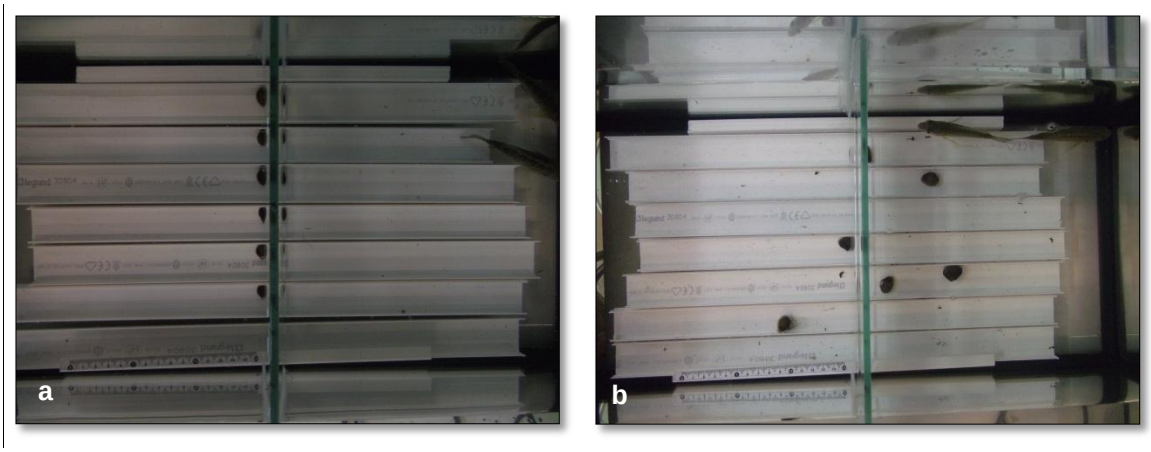


Figure 3 – Example of an aquarium during the experiment. (a) Beginning of the experiment; t = 0h; (b) End of the experiment; t = 24h. Notice the evolution of the position of the clams in each runway.

2.4 Statistical analysis

The effect of the fish diet (fish factor) and of the isolation from direct contact with the predator (net factor) on the mean distances (n = 3) traveled by *C fluminea* treatments was analyzed using a two-way ANOVA, independently for each light regime. Whenever a significant interaction between both factors was present, simple main effects of fish were analyzed, with and without the net, using the error term from the two-way ANOVA as the denominator of the F-tests (Quinn and Keough 2002). A Tukey test was then applied to

assign statistically significant differences between treatments. A significance level (α) of 0.05 was used in all analyses.

3. RESULTS

A graphical analysis of the mean distance traveled by the clams along the 24 h of the experiment suggests that the presence of the net inhibits somehow the activity of the clams, regardless the predator diet and the illumination conditions (figure 6). The spread of distances traveled when the net was present was very narrow (i.e., clams were largely immobile), while a wider range of distances travelled were found in the treatments without the net (figure 6). This is a generally consistent pattern even when encompassing the large individual variation (see Annex I for data on individual clams within replicate aquaria). This variability was mainly due to the treatment with fish fed with *Corbicula* (F_{cf}) – see Fig. 6. Illumination also seems to influence consistently the activity of the clams. Mean distances travelled by the clams, as shown in figure 6 (see also Annex I, for data on individual clams), denote both the lower activity in general and the lower variation between fish-factors treatments in the dark.

Although the apparent role of illumination and net protection modeling the clam's response, the mean distance traveled by *Corbicula fluminea* seems to be consistently smaller in the control treatments, i.e., when the predator challenge was absent (figure 6). Fish fed *C. fluminea* apparently enhanced the behavioral response of the clams either towards (preference; negative distances) or away from (escape; positive distances) the predator (figure 6, top panels). The opposite pattern in the travelling direction driven by the illumination conditions should be noticed: while in the dark clams seem to escape from the fish fed *C. fluminea*, under permanent illumination they exhibit an apparent preference towards the predator area of the aquarium. This is clear in 2 out of 3 replicate aquaria (see Annex I-A). Fish fed with chironomid larvae seem to be less effective in promoting changes in the clam's behavior than fish fed *C. fluminea* (lower mean travelled distances; figure 6-top panels).

Visual inspection of the dislocation of the clams in all test treatments at all assessment time-points suggested that the first 6 hours constituted the critical assessment time-period (figure 6). Hence, statistical analysis focused on the data retrieved after 2 h and 6 h of predator challenge. Figure 7 displays the data at the discrete time of 6 h post-challenge. A

significant interaction between fish and net factors was recorded at 6 h (table 1). This confirms our general observations (see above), which show that the response to the putative predator cue depended on the presence or absence of the protective net. Indeed, simple main effect analysis (stemming from the 2-way ANOVA) revealed no significant differences in the distance traveled by the clams across fish treatments when the net was present (lights on: $F_{2, 12} = 0.248$, $P = 0.784$; lights off: $F_{2, 12} = 0.036$, $P = 0.964$), but a significant effect of fish factor was noticed in the no-net treatment (lights on: $F_{2, 12} = 6.271$, $P = 0.014$; lights off: $F_{2, 12} = 10.686$, $P = 0.002$). Such effects were only perceptible at 6 h (interaction was not significant at 2 h; table 1); independently of the illumination conditions, 2 hours were not enough to promote significant differences in the dislocation of the clams.

The effects of fish-conditioned water when the net was not present were due to the treatment where fish were fed with *C. fluminea* (see Tukey homogenous groups, depicted as different letters in figure 7), as stated above. Although statistically significant in both illumination scenarios, the differences observed were completely opposite and perceptible at 6 h (figure 7). Under permanent illumination, clams seem to prefer the predator area in the aquaria, while they were found more prone to escape from the predator area in the dark. The magnitude of the clams' dislocation was higher under the constant light regime (figure 7).

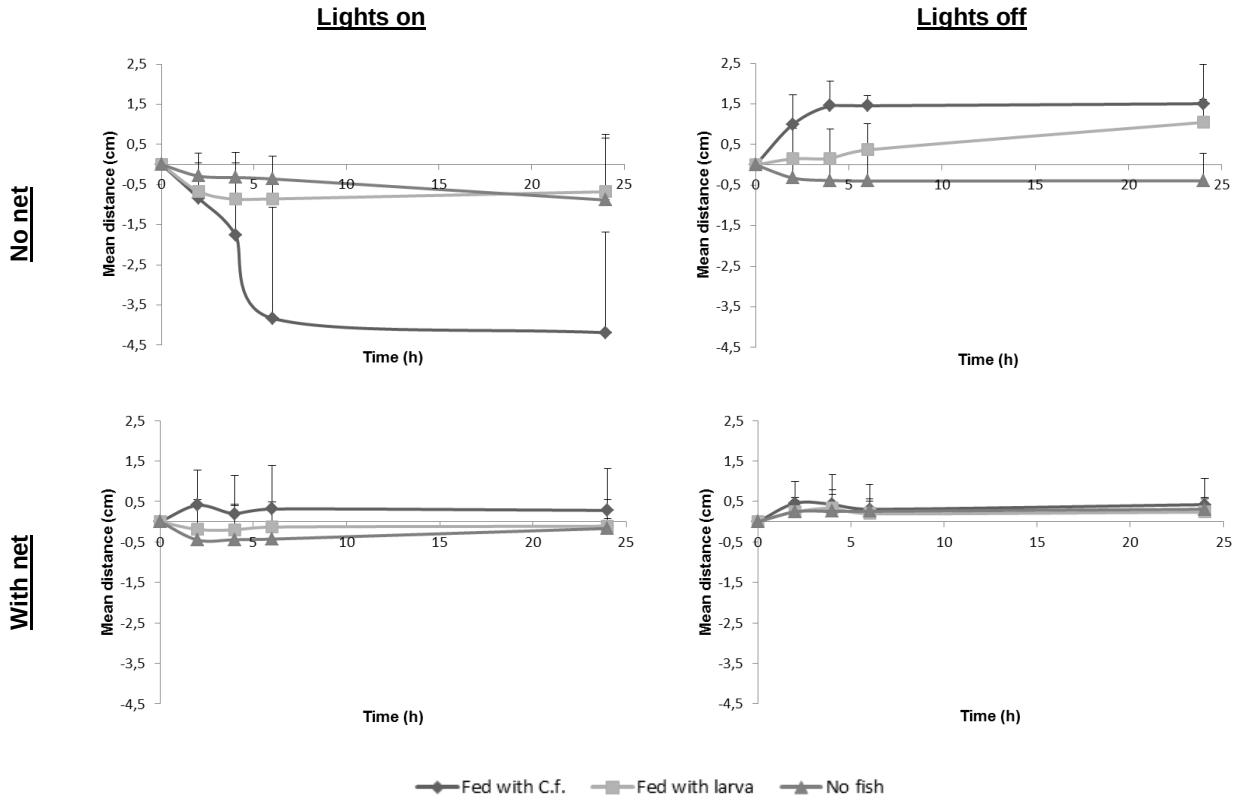


Figure 6 – Mean distance (cm) traveled by the clams challenged by the fish-conditioned treatments, under different illumination conditions (left versus right panel) and protected/unprotected from direct contact with the predator (top versus bottom panels), throughout the whole duration of the experiment (24 h). The graphics shown in the top panel correspond to aquariums A, B and C while those shown in the bottom panel correspond to aquariums D, E and F, as detailed in Annex I. Error bars represent SD.

Table 1 – Two-way ANOVA summary relative to the distances travelled by the clams following 2 h and 6 h of challenge by fish-conditioned water (fish factor), with or without direct contact between predator and prey (net factor), under permanent illumination or permanent darkness conditions. df — degrees of freedom.

Illumination conditions	Time-point	Source of variation	df	MS residual	F ratio	P value
Lights on	2 h	Fish	2, 12	0.0726	0.14	0.872
		Net	1, 12	1.2146	2.32	0.153
		Fish * Net	2, 12	0.7449	1.42	0.279
	6 h	Fish	2, 12	3.477	2.06	0.170
		Net	1, 12	11.541	6.84	0.023
		Fish * Net	2, 12	7.527	4.46	**0.036**
Lights off	2h	Fish	2, 12	0.9261	2.61	0.114
		Net	1, 12	0.0074	0.02	0.888
		Fish * Net	2, 12	0.4661	1.31	0.305
	6 h	Fish	2, 12	1.3924	5.71	0.018
		Net	1, 12	0.2175	0.89	0.364
		Fish * Net	2, 12	1.2227	5.01	**0.026**

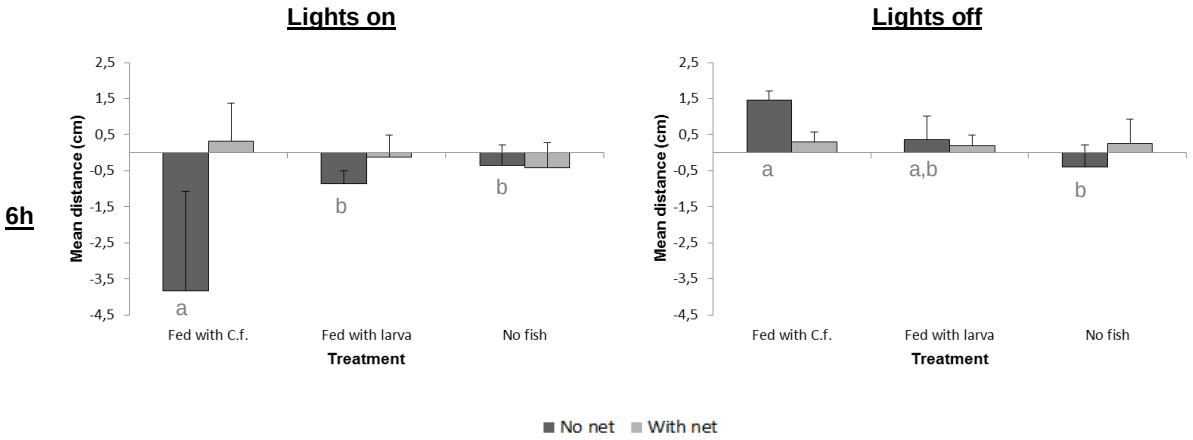


Figure 7 – Mean distance (cm) traveled by the clams challenged by the fish-conditioned treatments and protected/unprotected from direct contact with the predator (with net versus no net bars), under different illumination conditions (left- versus right-hand panel) on the 6th hour of the experiment (top versus bottom panels). The graphics shown in dark grey correspond to aquariums without net, while those in light grey correspond to aquariums with net. Positive values represent avoidance to fish. Different letters (a and b) represent significant differences between the treatments, according to Tukey test. Error bars represent SD.

4. DISCUSSION

The experiments made in this work revealed a larger tendency for active dispersal in clams exposed to *Lepomis gibbosus*. However, some contradictions aroused from the experiments, as the response from clams seems to depend on a number of factors (see below). Such contradictions hindered our ability to conclude on the potential of using fish (or infochemicals) as a biological agent to induce avoidance of the nuisance *C. fluminea*.

First, there was a clear effect of the presence/absence of the net on the obtained results. One hypothesis is that the clams seem to perceive the existence of a refuge and lie practically motionless, irrespective of predator presence and light regime. Some previous studies using other preys emphasize the importance of refuge under predator challenge:Turner and Montgomery (2003) demonstrated, using *Lepomis gibbosus*, that the behavioral response of the snail *Physa acuta* to the caged fish depended on their proximity to the fish, proving that the farthest the snail was from the fish, the less they used the covered habitat. This suggests a perception of danger by the snail, when exposed to a predator. Similarly, in our experiments, we expected that the response of clams to predators was

mediated by fish infochemicals. However, another explanation is possible: the net protects clams from being mechanically disturbed (e.g. poking, foraging attempts, involuntary strokes) by fish, and this mechanical disturbance could be the necessary stimulus for an antipredator response (e.g. escape, avoidance, etc.). Experiments made by Kobak et al. (2012) using *Dreissena polymorpha* and gammarids support this view of the antipredator defense activated by mechanical irritation; no responses to the infochemicals were observed when gammarids and mussels were separated by a mesh screen, similarly to our experiment. As we will see in the next paragraph, data do not fully support this.

One factor that supports the idea that *C. fluminea* does respond to an infochemical (kairomone), is the fact that the fish fed with *Corbicula* induced a more evident effect in the clam dislocation than when fish were fed with chironomid larvae (in both cases, this was true when the separator net was absent). This suggests an alarm response to dietary cues that are dependent to previous ingestion of prey. Literature refers that the most prevalent mode by which chemical cues determinate predator-prey interactions is by recognition of predator odors through kairomones (Kats and Dill 1998, Castro et al. 2007). After passing through the predator's digestive tract, the prey-specific cues and their metabolites remain detectable by living prey (Chivers and Smith 1998). These diet cues are supposed to be similar to injury-released alarm cues (Wisenden 2003). A major flaw of this idea is that we cannot explain the dislocation of the clams towards the predator, as occurred in the "lights on" treatment, without net. The fact that this was not consistent – there was an overall movement in the opposite direction observed in the "lights off" treatment – is even harder to explain.

Differences between lighting regimes were expected due to a) fish and clams are presumably less active during the night; b) clams cannot use visual cues to detect predators at night. Kobak and Nowacki (2007) found that the mussel *Dreissena polymorpha* exhibits a clear preference towards dark refugia and modifies its behavior according to light intensity, suggesting that illuminated sites are related to increased exposure to predators (Kobak 2001). Under strong light, this bivalve reduced its movements; we found the opposite pattern when the predator was present. Since the Asian clam is mobile, while zebra mussel is mainly sessile, it is possible that *C. fluminea* individuals were seeking a dark refuge. Indeed, clams were more active in the "lights on" treatment. It is just not clear whether there was some sort of purpose in the direction of their movement.

All these contradictory results may derive from a potential bias. Due to the failure, so far (e.g. Pohnert and Von Elert 2000, Lass and Spaak 2003), in the identification of the

chemical substances that comprise “fish kairomones”, there is no known way to measure the kairomones. Therefore, we cannot be sure that a kairomone gradient is established in the aquaria, or that this gradient is constant. In our experiments, there were small fluctuations in mean distances travelled from the 6th to the 24th hour, suggesting that such a gradient must have disappeared. From the 6th hour onwards, it is likely that the fish cue should dissolve evenly throughout the aquarium, thus ending the initial response. However, without chemical confirmation, there is no way of knowing. Because of this, inferences on the direction taken by the clams (i.e. towards or in the opposite direction of the fish area) may not be correct. Indeed, when detecting the fish kairomone, clams may respond by moving erratically as an initial fright or avoidance response (Côté and Jelnikar 1999, Wisenden 2003, Griffiths and Richardson 2006 called it flight); in this way, the direction which they take may be a product of chance rather than the result of a kairomone gradient.

So far, we have shown evidence that support both mechanical irritation and chemical cues as the mediators of antipredator responses. It is possible that both mechanisms coexist. Our results cannot clarify this: while the effect of net presence/absence clearly suggests that direct predator-prey contact is necessary to elicit antipredator response, the fact that clam movement was more pronounced in the treatment where fish were fed *C. fluminea* makes us suspect of a kairomone-mediated effect. Such contradictions make this study inconclusive on the effects of the studied fish on the avoidance induction in *C. fluminea*, thus leaving unanswered its potential use as a biological control method.

Our a priori objective was to assess the use of fish presence (or its kairomone) as a way to induce avoidance or flight behavior from *C. fluminea*, thus establishing a proof of principle regarding the use of fish to promote clam exclusion zones in sensitive areas of water-dependent infrastructures. While results do support predator-mediated responses from *C. fluminea*, their contradictory nature requires us to be cautious in extrapolating their utility as biological control methods.

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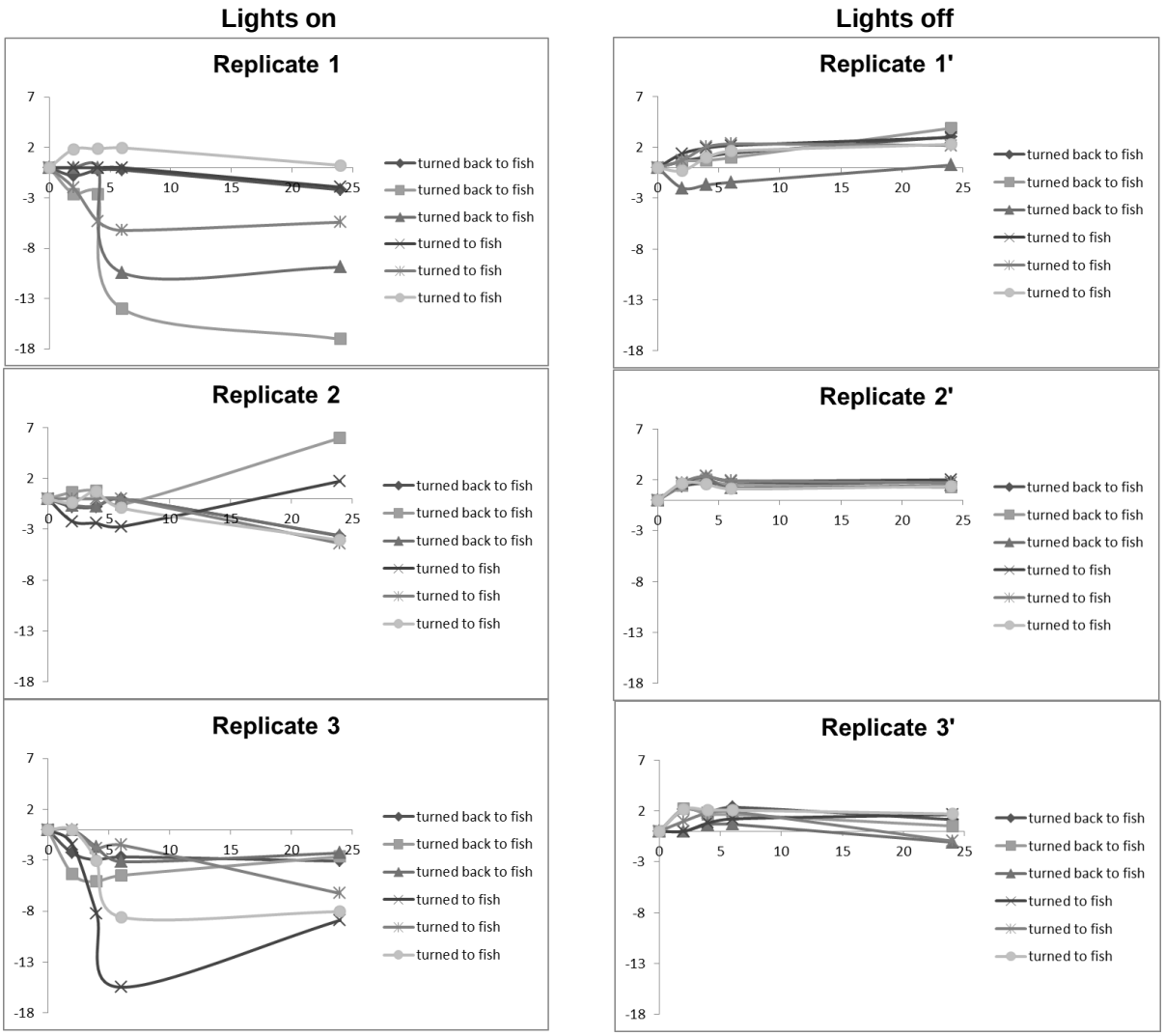
6. ANNEXES

Annex 1 – Distance travelled by individual clams in each replicate aquarium: Visual inspection of the travelling of the clams in all test treatments at all assessment time-points suggested that the first 6 hours constituted the critical assessment time-period (see the results section for further details)

A - Fish fed with *C. fluminea*
No net



B - Fish fed with larvae

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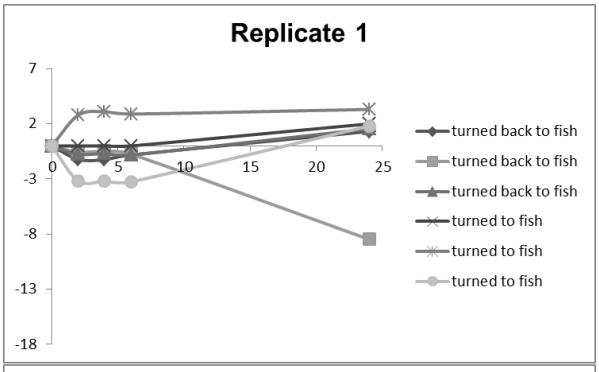

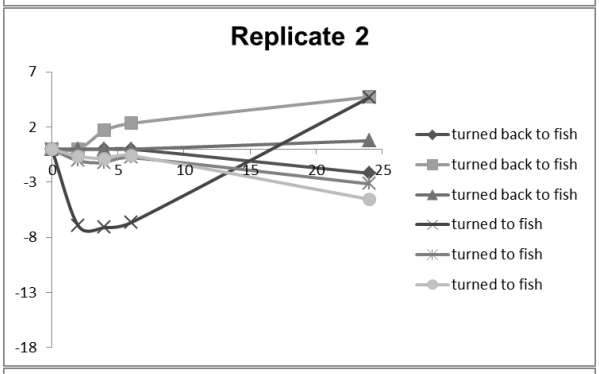

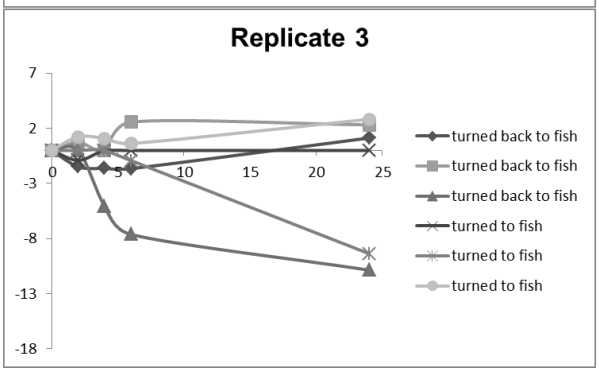

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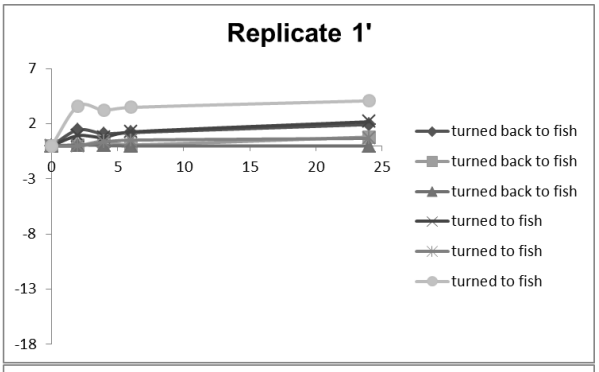
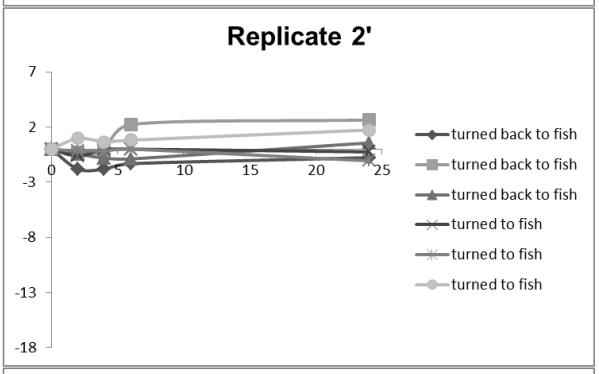
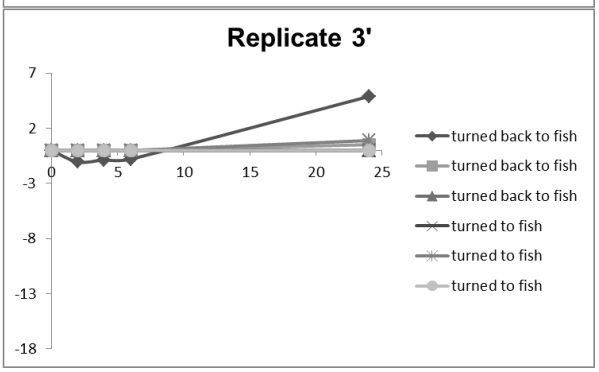


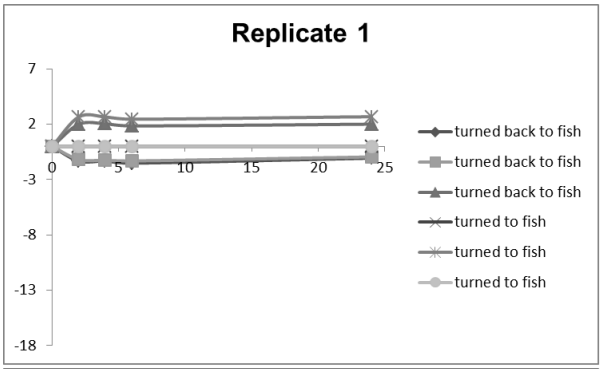


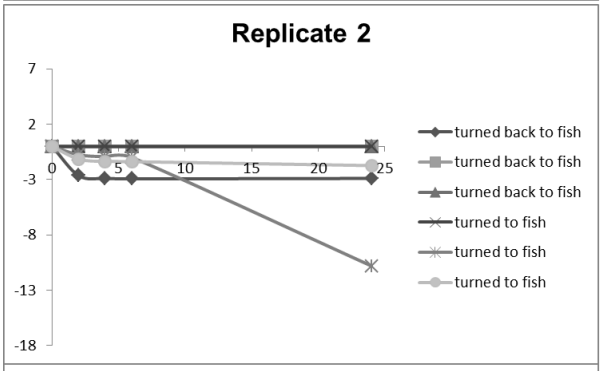


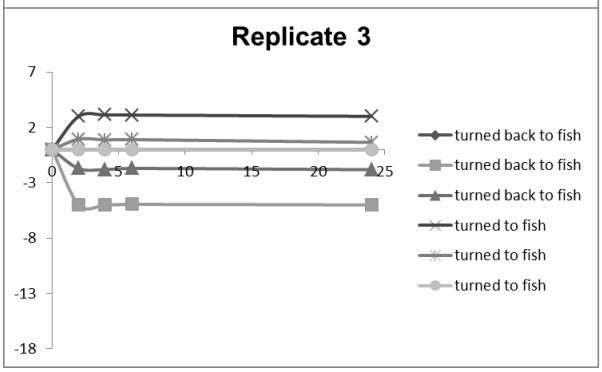
C - No fish

No net

Lights on







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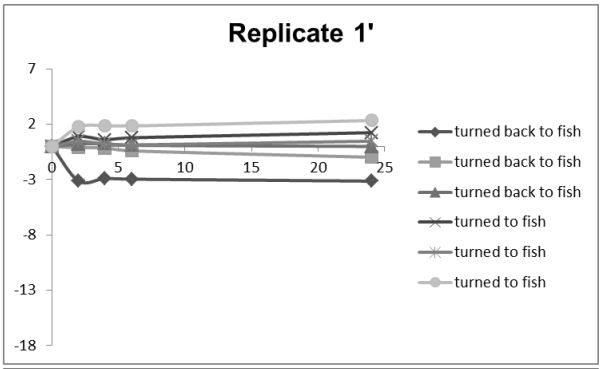
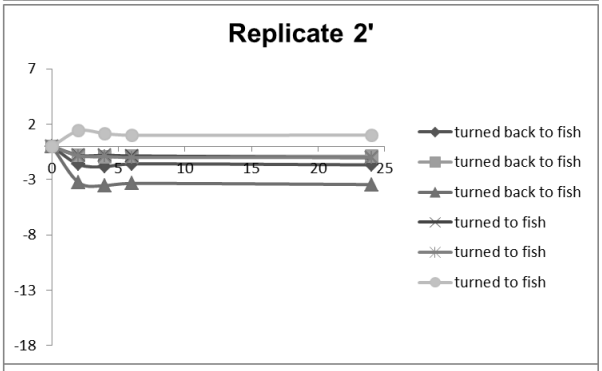
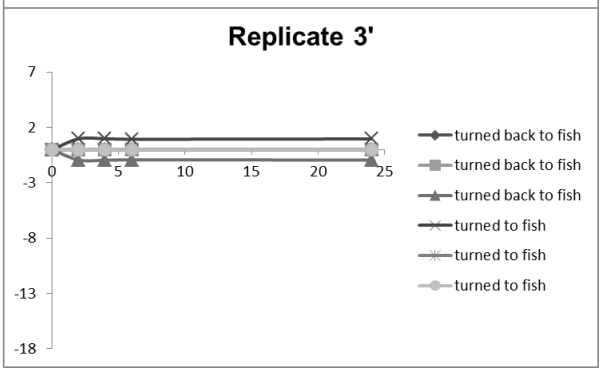


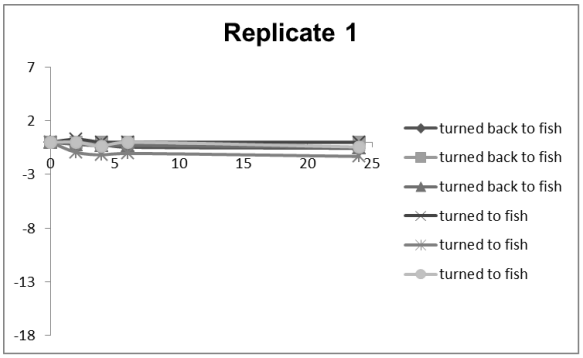


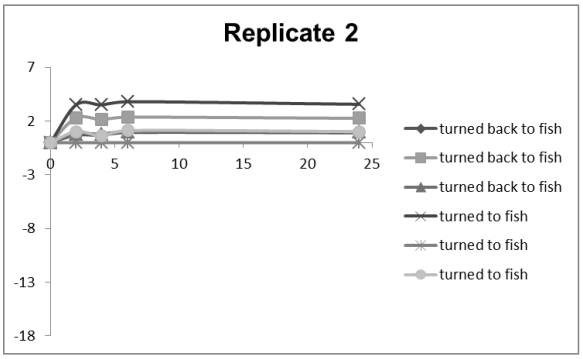


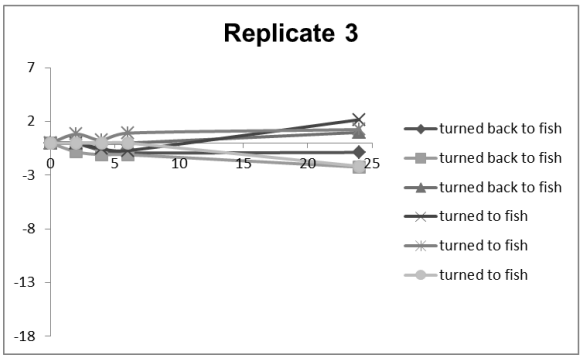
D - Fish fed with *C. fluminea*

With net

Lights on







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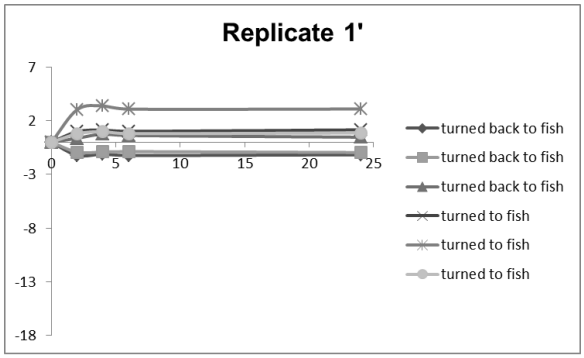

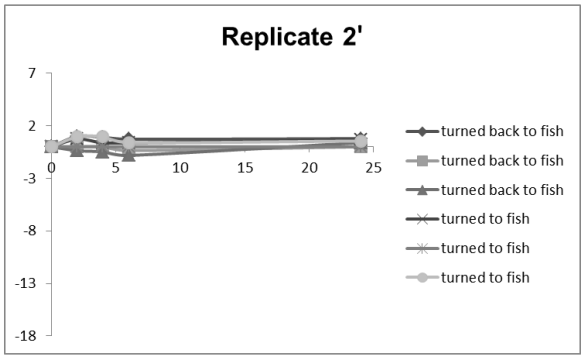

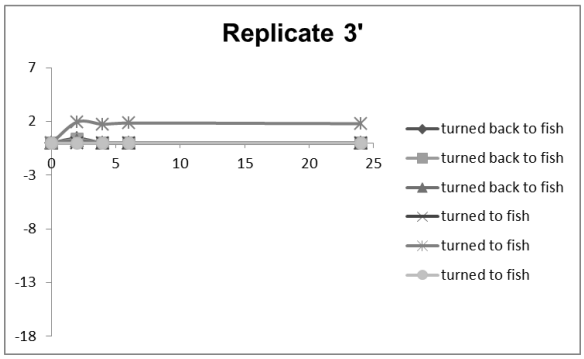

E - Fish fed with larvae
With net

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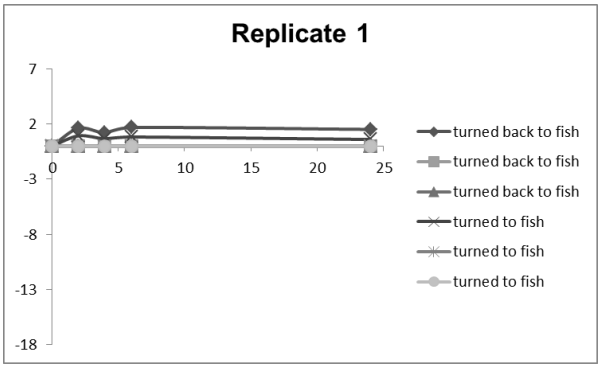

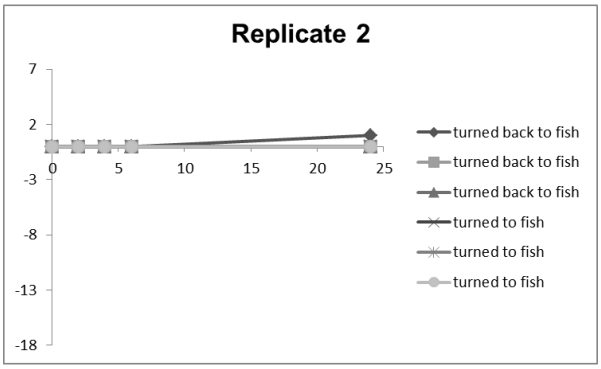

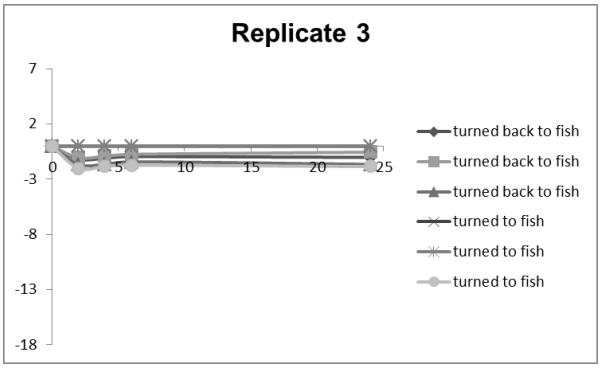

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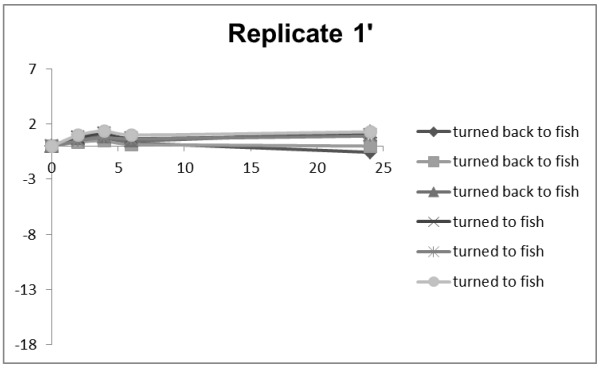
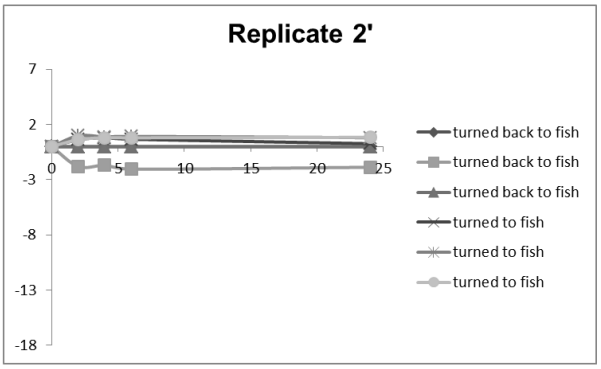
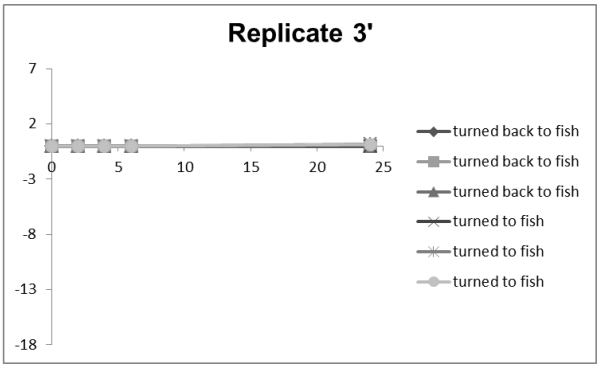
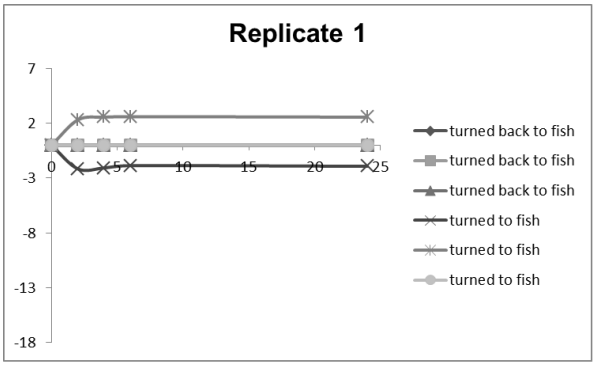


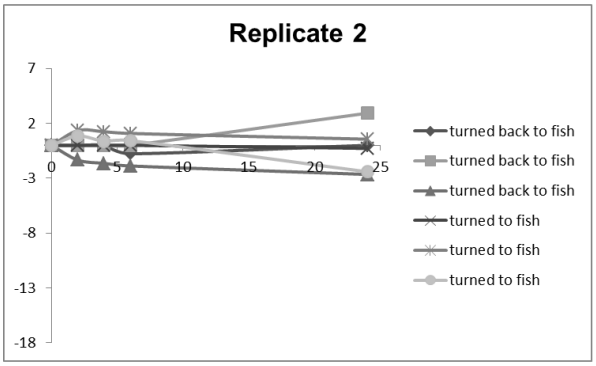


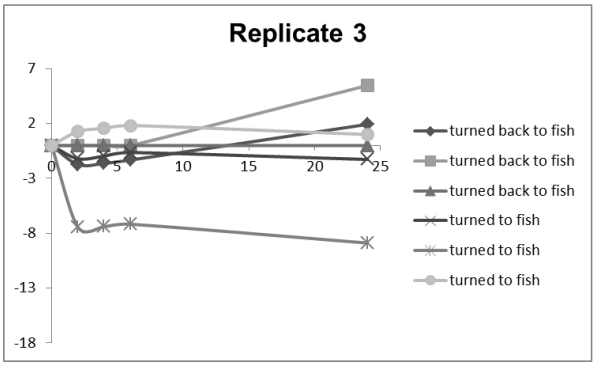


F - No fish
With net

Lights on







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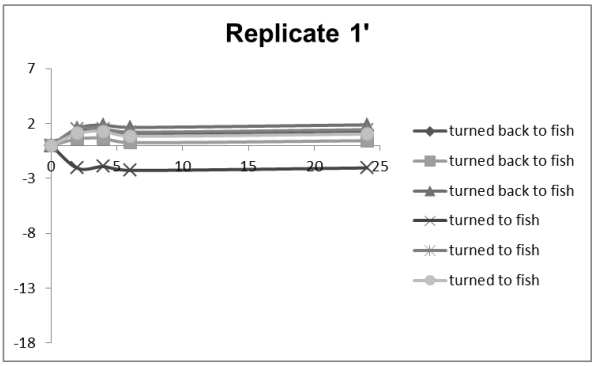

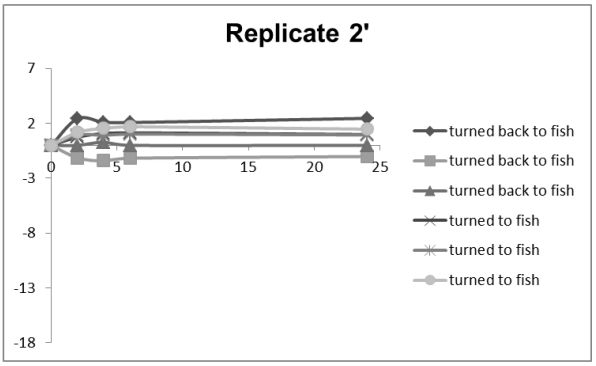

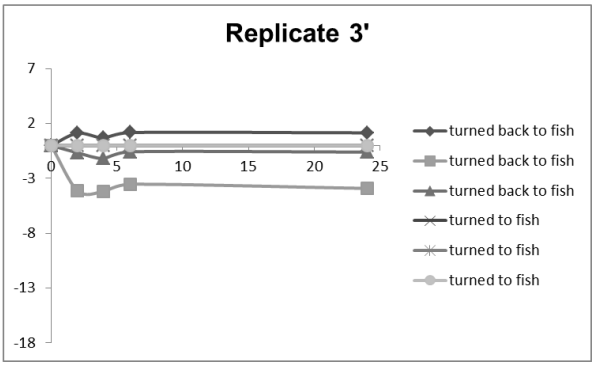

CONSIDERAÇÕES FINAIS

O presente estudo explorou o potencial de métodos diretos e indiretos de controlo da espécie invasora *Corbicula fluminea*, com recurso a experiências de predação e a experiências de evitamento. Face aos resultados apresentados no 1º capítulo, relativamente à eficiência da predação no controlo de populações de *C. fluminea*, as espécies de peixes em estudo revelaram pouco potencial como agentes predadores. No 2º capítulo demonstrou-se que a presença de *Lepomis gibbosus* induziu um efeito de dispersão em *C. fluminea*, apesar de essa dispersão depender de alguns fatores que resultaram em respostas contraditórias.

No âmbito da primeira experiência, apresentada no capítulo 1, consideraram-se peixes moluscívoros para representar o predador na análise das interações com *C. fluminea* enquanto presa. Nomeadamente, utilizaram-se o barbo, *Barbus bocagei*, e a perca-sol, *Lepomis gibbosus*. Ambos são apontados como potenciais predadores da espécie invasora na literatura, embora apenas para o barbo tenha sido evidenciada a atividade de predação sobre o bivalve no campo. A discussão acerca da utilização de peixes no controlo biológico de espécies de bivalves invasores demonstra algum consenso relativamente ao facto de que, mesmo que os peixes não consigam exterminar um invasor, podem contribuir para a manutenção das suas populações em baixos níveis de densidade (Garcia and Montalto 2006, Cantanhêde et al. 2008). Contudo, os nossos resultados, apesar de reconhecerem que os tamanhos dos predadores e das presas podem originar diferentes cenários de predação, demonstram níveis pouco ou nada significativos de predação, por *Barbus bocagei,* e efetivamente nulos por parte de *Lepomis gibbosus*. Experiências adicionais permitiram demonstrar que os níveis baixos ou nulos de predação observados serão causados essencialmente pelo efeito protetor da concha de *Corbicula fluminea*. Em suma, e relativamente à utilização potencial da predação por estas duas espécies de peixes como ferramenta de controlo biológico de *C. fluminea*, os resultados não foram promissores. No entanto, dadas as vantagens que o desenvolvimento de métodos de controlo biológicos baseados na predação poderia trazer no controlo de bivalves invasores, sobretudo onde o uso de outras soluções (p.ex. químicas) possa estar limitado, a investigação não se deverá esgotar nos primeiros passos dados no presente trabalho. Por um lado, é possível que as populações de peixes presentes no local onde foram recolhidos ainda não tenham desenvolvido a habilidade de predar *Corbicula fluminea*, como determinou Correia et al. (2005) em experiências de predação com *Procambarus clarkii* e *Corbicula fluminea*, apesar de a coexistência entre predador potencial e presa ter sido confirmada. Em artrópodes, o

aumento na eficiência do ataque está relacionado com a duração do tempo de aprendizagem (Lawton et al. 1974 in Usher et al. 1974). Em princípio, a presença de conchas de *C. fluminea* em 50% dos indivíduos de *Barbus bocagei* recolhidos demonstra que pelo menos esta espécie já reconhecerá a amêijoa como presa. No entanto, dada a elevada densidade de *C. fluminea* nos canais, não se pode descartar a hipótese de as conchas terem sido ingeridas acidentalmente, já partidas. Em testes futuros, com recurso a *B. bocagei* e *L. gibbosus*, poder-se-á clarificar este ponto assegurando que é fornecida uma dieta contendo *C. fluminea* ao peixe pelo menos durante um mês (conforme sugerido por Correia et al. 2005), para posteriormente se testar a eficiência da predação. Para estudos futuros, sugere-se a avaliação da eficiência de predação de *C. fluminea* por outros potenciais predadores, como o peixe-gato e o lagostim, ambos reportados como predadores moluscívoros (Covich et al. 1981, Magoulick and Lewis 2002), com as características anatómicas necessárias à predação de *Corbicula*. O lagostim *Procambarus clarkii* foi inclusive reportado como predador de indivíduos de *Corbicula fluminea* com <6 mm (Covich et al. 1981), e o lagostim *Orconectes spp.* como predador de mexilhão-zebra com <5 mm, pelo que a análise da predação nesta classe de tamanho da presa deve ser alvo de especial atenção em futuras experiências, a conduzir por exemplo com recurso ao lagostim presente nos canais de Mira - *Procambarus clarkii*.

Dada a ineficiência da predação pelas duas espécies de peixes testadas como potencial método de controlo direto de *Corbicula fluminea*, considerou-se a possibilidade de explorar o desenvolvimento de métodos de controlo biológico indireto da espécie. Neste sentido, avaliou-se a existência de comportamentos de evitamento de *Corbicula fluminea* face à presença de um potencial predador no sistema, neste caso *Lepomis gibbosus*. Os resultados indicam que as amêijoas conseguirão detetar a presença de um elemento adicional no sistema (neste caso, o predador). Vários fatores (presença de refúgio, condições de iluminação e dieta do predador) demonstraram condicionar o movimento da presa, o que dificulta a elaboração de conclusões acerca do potencial deste tipo de alternativa no controlo biológico de *C. fluminea*. O presente trabalho deixa assim em aberto uma série de questões que merecem futura atenção. Propõe-se portanto a realização de experiências de evitamento com recurso à espécie *Barbus bocagei*, que não foram possíveis de realizar devido a limitações de espaço em laboratório e ao tempo limitado para a realização da dissertação. Dentro dos métodos indiretos, parece pertinente explorar experiências de evitamento realizadas em ausência de luz e de abrigo, com diferentes dietas

do predador como variável. Primeiramente julgou-se que as amêijoas teriam a perceção da presença do predador através do reconhecimento de cairomonas, e responderiam a essa perceção deslocando-se no sentido contrário ao do predador. No entanto, a inconsistência dos resultados não permite confirmar este padrão hipotético. Assim, torna-se relevante proceder a uma análise e caracterização mais detalhada da resposta comportamental de *C. fluminea* à presença de predadores, por exemplo, explorando a emissão de infoquímicos por outras espécies predadoras e não predadoras de *Corbicula*. Na continuidade deste estudo, parece também pertinente explorar sistemas em que a capacidade de manter o gradiente de cairomona seja mais eficiente. Na sequência da análise temporal das experiências realizadas, também deverá ser realizado um teste de maior duração, que permita estabelecer um gradiente mais prolongado de exposição do infoquímico a *C. fluminea*. Dado que também o fator luz influenciou a movimentação de *C. fluminea*, será necessária também uma averiguação mais detalhada dos efeitos provocados pela iluminação, de modo a perceber se em condições de luz, a sombra ou a perturbação mecânica geradas pelo peixe poderão condicionar a resposta da amêijoa, ou se existe realmente atuação de um infoquímico.

O desenvolvimento de um método capaz de beneficiar do comportamento de evitamento anti-predador por parte do invasor será útil na produção de uma zona de exclusão, livre de *C. fluminea*, com potencial aplicação em estruturas subaquáticas. A melhor compreensão dos fatores que condicionam o comportamento de *C. fluminea*, associada ao seu comportamento de evitamento, poderão ser importantes ferramentas para prevenir o entupimento de bombas de alimentação de estruturas industriais, assim como reduzir a progressão do invasor para zonas mais sensíveis das estruturas subaquáticas. Os impactos de *C. fluminea* referidos nesta dissertação – ecológicos, económicos ou industriais – remetem para a necessidade de continuar a procurar um método de controlo desta amêijoa. Neste contexto, é importante o desenvolvimento de trabalhos como este, que têm o potencial de estabelecer provas de princípio (p. ex. evitamento na presença de infoquímicos de predadores) que, ulteriormente, possam dar origem a ferramentas inovadoras de controlo de invasores, passíveis de ser aplicadas em larga escala.

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